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EFFECT OF VAGUS STIMULATION ON THE BLOOD FLOW THROUGH THE KIDNEY AFTER SECTION OF THE CORRESPONDING SPLANCHNIC NERVE

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Asher and R. G. Pearce have recently demonstrated the existence in the vagus nerve of fibers having a secretory influence on the renal function. Decerebrate animals (cats and dogs) were employed in these experiments, the vaso-motor control exercised on the renal vessels through the splanchnic nerve being removed on the left side by cutting the nerve just above the adrenal gland. The vagi, after cutting them in the neck, were stimulated below the level of the heart. The kidney on the opposite (right) side was at the same time completely isolated from the nervous system by carefully cutting all the nerves surrounding the renal vessels. By observing the flow of urine from the denervated kidney simultaneously with that from the kidney on the side in which the vagus was stimulated the influence of changes in the general blood pressure on the urinary flow was allowed for.

Although previous observers have been unable to detect any influence of the vagus on the blood flow through the kidney by oncometric or strumuhr methods, when the splanchnic nerves are intact, yet it remains possible that vaso-dilation might occur under the conditions outlined above, and that the increase in

blood flow was the cause of the diuresis. Not only might differences in the effect of stimulation of the vagi depend on the presence or absence of splanchnic control, but it must be remembered that there may be an increase in the blood flow of an organ without any measurable change in its volume.[1]

For these reasons I believe that it is necessary to carefully investigate the flow of blood through the kidney under the conditions that were present in the experiments of Asher and R. G. Pearce.

METHODS

In the majority of the experiments the same technique was used (as far as possible) in the preparation of the animal as that employed by Asher and R. G. Pearce. One difficulty of such a procedure lies in the fact that considerable shock may have supervened before all the necessary steps in the operation have been completed. To obviate this in a number of experiments the animal was prepared three days before the actual experiment by a preliminary operation which consisted in cutting the left vagus in the neck, the splanchnic nerve on the left side above the adrenal gland and the nerves of the right kidney. Not only had the shock of the operation passed by this time but the cardiac fibers in the vagus had degenerated to such a degree that cardiac inhibition was no longer caused by vagus stimulation. In these cases ether anaesthesia was used in place of the decerebrate preparation.

Two methods were employed in the estimation of the rate of blood flow through the kidney. The first was the oncometric method. In these cases the kidneys were placed in hinged brass shells in which they were completely surrounded with a tube of the very thinnest rubber, and which communicated by means of tubing with a volume recorder. The tubes were then filled with air under the proper tension to record the pulse and respiratory waves of the kidney on a kymograph. Such an oncometer is easy to apply and gives a most excellent tracing.

[1] Burton-Optiz and Lucas: Journ. Exper. Med., 1911, xii, 308; Asher and R. G. Pearce, Zeit. f. Biol., 1913, lxiii, 7.

The second was a modification of that described by Barcroft and Brodie.[2] After preparing the animal for the experiment on kidney secretion as described above, the aorta and the vena cava are ligated below the kidneys, also ligatures are placed on the right renal vessels, the adrenal vessels, the left spermatic vein, and any veins that empty into the vena cava in the region of the kidneys. A loose ligature is placed about the vena cava above the left renal vein and a large paraffined cannula inserted in the vena cava below. This cannula is attached to a receiving vessel into which the blood may flow without resistance, clotting being hindered by using a small amount of huridin or extract of leach heads in saline. The receiver is connected by tubing with a recording instrument.

The recorder used in the earlier experiments was one devised by Professor Brodie for whose permission to use it in the present research I am much indebted. Professor Brodie will describe the apparatus in the near future; suffice it to say here that it is a very simple and sensitive form of piston recorder which accurately records as an arc on a kymograph the amount of air displaced during the filling of the receiver with blood. The recorder is calibrated and a certain height chosen on the arc represents a definite amount of fluid in the receiver. By measuring the time which elapses from the beginning to the height chosen on the arc the rate of outflow can be estimated. To obviate the necessity of measuring the arcs, which to do accurately requires great care and is moreover a time consuming process, the method was modified by causing the lever during its ascent to close and to open an electric circuit in which there is an electric signal which records on a kymograph. By changing the relative position of the electric contacts the instrument may be made to register varying amounts of blood, which is determined for each exact position of calibration.

The accuracy of the instrument was tested by allowing water to flow into the receiving flask from a Mariott bottle, which permitted a constant outflow. The results obtained by these measurements proved that the instrument measured accurately

[2] Barcroft and Brodie: Journ. Physiol., 1908, xxxii, 18.

the outflow of fluid inasmuch as the record on the kymograph was exactly the same in all the trials. The amount of fluid which it was necessary to introduce into the receiving bottle in order to make the first contact was 5 cc. and the amount of fluid represented by the period between the make and the break signal was 11.25 cc. as determined by actual tests.

I later found that a little modification of the recording instrument would reduce the inertia and the friction. This consisted of a thin walled parchment thimble coated with shellac inverted over a small glass tube which perforated the bottom of, and extended level to the top of a larger glass tube which was filled with water. By placing a small rim of cork about the bottom of the parchment thimble the amount of resistance offered to the raising of the tube can be reduced to almost nothing, and the slightest force will cause the tube to rise. The small inner tube is connected with the receiving bottle as in the former instrument. Any displacement of air in the bottle will cause the parchment tube to rise and to make and break the electric signal as in the first instrument. The advantage of the modification lies in the lessened resistance it offers to the outflow of the blood. The results with this instrument are however similar in the main to those of the older instrument.

In order to measure the outflow of blood the clip closing the vena cava at the entrance to the cannula is opened and the loose ligature which lies about the vena cava above the renal vein is tightened sufficiently to close the vein. The blood by these measures is diverted into the receiving bottle. At the close of the observation the blood is allowed to flow back into the circulation by raising the bottle. Great care is taken to eliminate any possible resistance to the outflowing blood, by keeping the receiving bottle at level of the kidney.

RESULTS

1. By the oncometer

In a series of five experiments on decerebrate dogs there was not the slightest evidence that any increase in kidney volume

occurred as a result of vagus stimulation. It was not attempted to take specimens of urine while the oncometers are in place because of the well-known difficulty of obtaining satisfactory excretion, for some time at least, after placing a kidney in an oncometer. In one of the experiments the chart of which is given below, however, the effect of stimulation on the excretion was determined before starting the experiments on volume.

Experiment, October 23, 1913. Male dog, weight 9 kgm. decerebrate, artificial respiration, electrodes placed on vagi in thorax below the heart, the left splanchnic severed, and the right kidney denervated and the ureters catherized. The blood pressure was recorded from the carotid artery. During a resting period of ten minutes the denervated kidney secreted 2 cc. and the left kidney 1.5 cc. During a period in which the vagi were stimulated the denervated kidney secreted 1.9 cc. and the stimulated kidney 2.3 cc. The blood pressure remaining at 100 mm. Hg. during the two periods. Immediately following this period the oncometers were quickly placed in position with as little damage to the kidney as possible. Changes in volume were recorded by volume recorders of equal size, 16 mm. height of arc on the tracing equaling 1 cc. of air displaced in the oncometer. The following curve is compiled from measurements of the tracing, the original tracing is too involved for suitable reproduction.

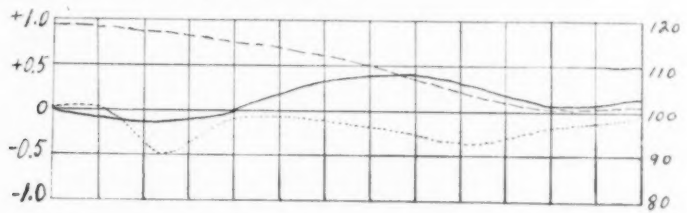

Fig. 1. Curve compiled from tracing of oncometers, October 23, 1913: Right kidney denervated, left splanchnic cut, vagi stimulated below level of heart. The ordinates on left indicate cubic centimeters increase or decrease in kidney volume; on the right, arterial blood pressure; the duration and time of stimulation are indicated by heavy lines on abscissa. Continuous line, volume of left kidney. Dotted line, volume of right kidney. Broken line, arterial blood pressure.

It is seen that the small changes that were present in the oncometric tracing show no dilation of the kidney due to vagus stimulation.

The above experiment is typical of the other four of this series. Inasmuch as only negative results were obtained by the above methods, four experiments on dogs which had been prepared by the preliminary operation described on page 152 were done. But the results were the same for in none of them was there any evidence of change in kidney volume as a result of vagus stimulation.

Such a result in a partially degenerated nerve could not however be taken as evidence for the independence of renal dilator and secretory fibers unless it can be shown by actual experiment that the latter are active under the conditions present. That they are active is shown by the two following experiments.

Experiments, December 12–13, 1913. The animals, both male dogs, weighing about 10 kilos, were subjected to the preliminary operation three days before the actual experiment. With the object of securing a copious urine secretion 1 gram of phlorhizin was administered subcutaneously four hours before the experiment. In this, the animals were anaesthetized with ether and the ureters catherized with small cannulae to which were attached 1 cc. pipettes, graduated in 1/100. Each period of observation lasted ten minutes during which the peripheral end of the cut vagus was stimulated at intervals of one minute. The blood pressure was recorded from the carotid artery. In neither experiment did the stimulation of the vagus have any effect on the heart. The following table records the results, which are also plotted as curves in figures 2 and 3.

TABLE 1

The effect of stimulation of a partially degenerated vagus nerve, in a dog whose splanchnic nerve on the left side had been cut some days previously

EXPERIMENT	PERIOD 10 MINUTES	URINE LEFT KIDNEY	URINE RIGHT DENERVATED KIDNEY	BLOOD PRESSURE
		cc.	*cc.*	*mm.*
December 12, 1913	1—normal	0.50	1.16	120
	2—stim.	0.81	0.90	120
	3—normal	0.42	0.66	100
	4—normal	0.36	0.65	100
	5—normal	0.11	0.65	100
	6—stim.	0.20	0.13	75
December 13, 1913	1—normal	1.87	3.00	120
	2—stim.	1.50	1.00	80*

* Blood pressure fell about the end of the fourth minute of the period and very little urine was secreted the rest of the period.

The following curves are compiled from the above table.

From these results the conclusion may be drawn that the secretory fibers to the kidney are not completely degenerated three days after section of the vagus, and that they can still functionate when stimulated.

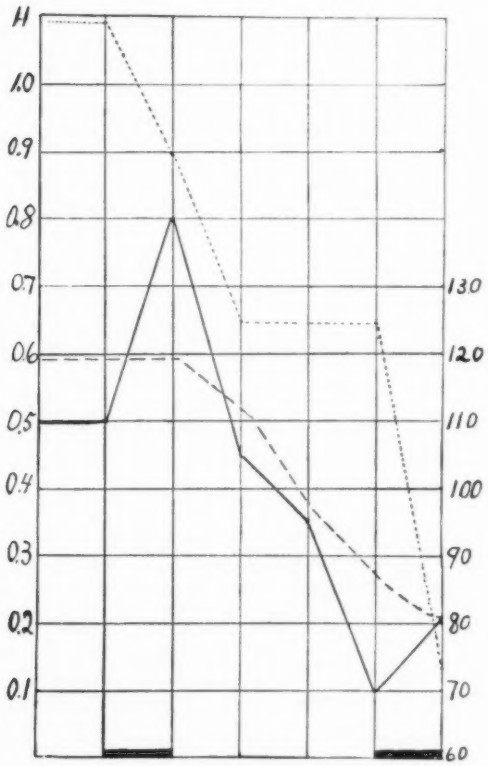

Fig. 2

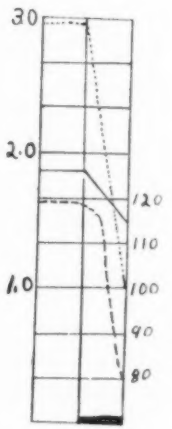

Fig. 3

Figs. 2 and 3. Dogs, December 12 and 13 respectively, 1913. Continuous line, left kidney; dotted line, denervated right kidney; broken line, arterial blood pressure. Ordinates on left indicate cubic centimeters of urine; on right, arterial blood pressure. Spaces left to right equal ten minute periods; time and duration of stimulation are indicated by heavy lines on abscissa.

2. By actual measurement of the blood flow through the kidney

Five experiments were performed on normal dogs under ether anaesthesia, four on decerebrate dogs and three on dogs with partially degenerated vagus. Greater than the normal variations in blood flow occurred in none of these experiments on stimulation of the vagus.

TABLE 2

Experiment to show the effect of splanchnic stimulation on the blood flow through the left kidney

NORMALS	STIMULATION SPLANCHNIC	NORMALS	STIMULATION SPLANCHNIC
3.05	3.38 coil at 20 cm.	3.38	1.1 coil at 9 cm.
3.05	3.38 coil at 20 cm.	3.38	0.59 coil at 6 cm.
	3.38 coil at 15 cm.		0.63 coil at 6 cm.
	3.05 coil at 10 cm.		0.43 coil at 6 cm.

The above observations were made in the above order, at intervals of about ten seconds. The blood pressure was not affected until the coil was at 10 cm. when a small rise in blood pressure occurred. At 9 cm. there was a marked rise in blood pressure.

As a test of the reliability of the method the following experiment in which the splanchnic nerve was stimulated with currents of varying strengths is given.

Experiment, March 10, 1914. Female dog, weight 10 kgm. Ether anaesthesia, left splanchnic cut and the peripheral end placed on electrodes. The blood flow of the left kidney was recorded. Blood pressure from the carotid. Blood flow given in cubic centimeter per second.

The above experiment would indicate that the method is applicable for recording slight changes in the blood flow through the kidney.

Tables Nos. 3 and 4 are typical records of experiments done on dogs under ether anaesthesia, and tables 5 and 6 are experiments on decerebrate dogs.

Experiment, March 11, 1914. Experiment to test the effect of vagus stimulation on the blood flow through the left kidney, after section of the left splanchnic above the adrenal gland.

TABLE 3

Experiment March 11, 1914

	NORMALS	VAGUS STIM. COIL AT 20 CM.	NORMALS	VAGUS STIM. COIL AT 10 CM.	NORMALS
	6.77	6.62	7.11	7.4	7.4
	6.62	* (8.0)	7.11	7.4	7.6
	6.62	7.11	7.4	* (9.3)	7.5
	7.4	7.11	6.24	7.4	
		7.11			
Average...	6.85	6.99	6.96	7.4	7.5

* These observations appear so far out of line that it is probable that they are incorrect. In none of the other experiments have such irregularities occurred.

TABLE 4

Experiment March 12, 1914

	NORMAL	VAGUS STIMULATION	NORMAL	VAGUS STIMULATION
	4.9	4.5	4.7	4.9
	4.7	4.3	4.9	4.7
	4.9	4.9	4.9	4.7
	4.5	4.7	4.9	4.7
		4.7		4.9
				4.9
Average	4.7	4.6	4.8	4.8

Male dog, weight 17 kgm. Ether anaesthesia, left splanchnic cut, vagi stimulated in thorax below the heart, blood flow of left kidney recorded by method described above. Blood flow given in cubic centimeters per second. Observations made every 15 seconds during each period.

Experiment, March 12, 1914. The same manner of preparation as the experiment in table 3. Male dog, weight 9 kgm. Blood pressure 115 mm. Hg. Flow given in cubic centimeters per second. Observations every fifteen seconds during each period.

Tables 5 and 6 are experiments on decerebrate dogs.

Experiment, March 13, 1914. Female dog, weight 8 kgm. Ether anaesthetic, decerebrated, left splanchnic cut above adrenal gland, vagi stimulated below the heart, blood flow from left kidney. The blood pressure was 100 mm. Hg. during the first period, but fell to about 80 mm. Hg. during the beginning of the second period, at which level

TABLE 5

Experiment March 13, 1914

	NORMALS	VAGUS STIMULATION	NORMALS	VAGUS STIMULATION
	2 5	2.56	2.74	2.56
	2.5	2.56	2.63	2.74
	2.6	2.50	2.56	2.63
			2.74	
Average...	2.53	2.54	2.66	2.64

TABLE 6

Experiment March 18, 1914

	NORMALS	VAGI STIMULATION	NORMALS	NORMALS	VAGI STIMULATION	NORMALS
	2.27	2.18	2.27	1.39	1.40	1.42
	2.32	2.20	2.18	1.39	1.40	1.42
	2.32	2.18	2.12	1.40	1.41	1.42
			*(2.09)	†		
Average...	2.30	2.22	2.25	2.39	1.40	1.42

* Blood pressure fell to 60 mm. Hg.

† Blood pressure fell to 50 mm. Hg.

it remained. Blood flow in cubic centimeters per second. Observations fifteen seconds apart.

Experiment, March 18, 1914. New form of recorder used, which measured 10 cc. blood at each observation. Male dog, weight 7.5 kgm Decerebrated, left splanchnic cut, vagi stimulated below the heart, blood flow from left kidney. Blood pressure at start of the experiment 80 mm. Hg. Blood flow in cubic centimeters per second.

If the two high figures found in the experiment recorded in table No. 3 are excepted, there is not a single indication in any experiment here tabulated or in the series done, which speaks for a dilator action of the vagus on the blood vessels of the kidney.

That these observations were not made during periods when there was known to be an increase in urine formation during vagus stimulation is to be regretted, since it may be said that since the experiments showed no increase in the rate of blood

flow it is possible that they also showed no change in the rate of urine secretion. In view of the difficulties of the experiment and the sensitiveness of the kidney to changes in its blood flow and to trauma, which prevent many experiments on the influence of the vagus on urine secretion from being successful, I did not attempt to make observations of the two phenomena simultaneously.

It may be presumed however that if there are vaso dilators in the vagus of sufficient power to account for the increase rate of urine excretion following vagus stimulation they would have appeared under the conditions that were present in the experiments.

Barcroft and Brodie[3] have shown that an increase in the urine formation is not necessarily accompanied by an increase in the rate of blood flow. The above experiments which confirm the observations of previous observers that the vagus is without effect on the blood supply of the kidney support the belief held by Asher and R. G. Pearce that the vagus has a true secretory influence on the kidney.

RÉSUMÉ

Since it was thought possible that the increase in urine formation found by Asher and R. G. Pearce to follow stimulation of the vagus nerve after section of the splanchnic nerve, might be due to an increased rate of blood flow through the renal vessels, the present investigation was undertaken.

It is shown by experiments on dogs under the same experimental conditions as those present in the experiments referred to, that no vaso-dilation occurs. These observations were made partly by oncometric measurements, and partly by actual measurements of the blood flow from the renal vein. For the latter purpose a modification of the Barcroft-Brodie method of measuring the blood flow is described.

The author wishes to express his thanks for the kind advice and criticism given him by Prof. J. J. R. Macleod.

[3] Journ. Physiol., xxxiii, 1905, p. 18.

THE REVERSIBILITY OF THE GEOTROPISM OF ARENICOLA LARVAE BY SALTS

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I. INTRODUCTION

In 1893 and 1904, Loeb discovered that Polygordius larvae, various copepods, Gammarus pulix, and Cyclops, which are negatively heliotropic, or indifferent to light, could be made positively heliotropic by salts, acids, etc. (10, 14). Since then, Holmes (3–4) Minkiewicz (27), Mast (24), A. R. Moore (29–31), Ewald (1) and others have shown various examples of the reversibility of heliotropism in animals by chemicals.

Anne Moore made an attempt by means of salts to reverse the geotropism in Paramecium. But she states that her results were "not very satisfactory" (28, p. 241). The writer has re-

peated her experiments, and is convinced that her results can by no means be called a reversal of geotropism. In the present paper, the writer shows that the negative geotropism of the larvae of Arenicola cristata is reversible by calcium and magnesium salts.

The experimental work was done partly under the direction of Prof. Ralph S. Lillie in the physiological department of the Marine Biological Laboratory at Woods Hole, Mass., during the summer of 1913. I wish here to acknowledge my indebtedness to Prof. Ralph S. Lillie and Prof. Elias P. Lyon for their valuable suggestions and criticism of the work and manuscript. My thanks are due, also, to Prof. Frank R. Lillie who gave me the privilege of occupying a table in the Laboratory during my stay.

II. MATERIAL AND METHODS

1. *Material.* The larvae of the marine annelid, Arenicola cristata, were used for this work. The larvae were obtained from the egg-strings. These were exposed to light in the laboratory for two or three days in dishes of sea-water together with some Ulva for aëration, as R. S. Lillie advises (6, p. 58). The free-swimming larvae just after they have left the strings at a "swarming stage" are remarkably favorable for work of this kind.

The larva at this stage is about 0.3 mm. long and 0.1 mm. in diameter at the anterior ciliary ring, which is the widest region of the animal's body. The larva swims spirally as Paramecium does. The reaction of the larva to gravity is far more pronounced than that of Paramecium.

2. *Methods.* The methods of experiment were very simple. At each trial, two 50 cc. graduates, one for experiment in darkness, and the other in daylight, were used. The desired amount of natural sea-water was poured into each graduate, and then the desired amount of the isotonic salt solution used was added to it, with vigorous shaking. After the disappearance of air bubbles, Arenicola larvae (taken with a small pipette from a very dense aggregation), were placed in each of the graduates. One gradu-

ate was placed under a dark cover-box as soon as possible and the other one on a table in front of the west window. Light came horizontally and in part from above. The readings were mostly made from the one on the table. At certain intervals, the one in the dark box was observed, as indicated in tables.

The time of observations was limited to about 30 minutes. The stronger the solution used the shorter was the time found to be necessary to produce the change in reaction. After each experiment the contents of each graduate, with the exception of 2 cc., were poured into a finger bowl for observation of the animals' life-endurance in the solution. To the remaining 2 cc. of each solution, 48 cc. of sea-water were added, and the graduates were replaced in their original positions for observation of the re-reversibility of geotropism, as well as of heliotropism.

The salt solutions, approximately isotonic with sea-water, used in all of the following experiments, were 0.53 m. for NaCl and KCl, 0.35 m. for $MgCl_2$ and $CaCl_2$, and 0.47 m. for $MgSO_4$.[1] The calculation of the isotonic concentration of all solutions was based on Garrey's results (2).

III. EXPERIMENTS

In preliminary tests, the writer found that Arenicola larvae in sea-water rendered hypertonic by the addition of NaCl or KCl became negatively heliotropic and that these negative animals swam downward. In this case, however, the downward swimming of the larvae was found not to be a gravity effect, because in the dark they stayed at the top. The addition of these two salts to the sea-water, therefore, had no effect on the geotropism of the larvae.

Arenicola larvae in sea-water rendered hypertonic by the addition of $CaCl_2$ or $MgCl_2$ became positively geotropic and swam downward in a typical manner. In darkness, they swam downward just the same. In order to test, therefore, whether the reversal of the normal negative geotropism of the larvae in

[1] $MgSO_4$ isotonic with sea-water should be 0.9 m. instead of 0.47 m. The writer's calculation was wrong (1914).

$CaCl_2$ and $MgCl_2$ was due to an osmotic or a specific chemical effect, the following series of experiments were conducted with isotonic solutions.

1. The effect of sodium chloride

As already stated, addition of NaCl to sea-water did not reverse the normal negative geotropism of the larvae. However, mixtures of isotonic NaCl solution and sea-water were found to reverse the positive reaction of the larvae to light. These negative animals did not stay very long at the side of the graduate farthest from the source of light, but swam downward at the side. In the dark, however, they stayed at the top. The downward swimming of the negative animals, therefore, was not a gravity effect, but seemed to be the result of a rather unstable negativating effect of light, or due to light coming partly from above.

Beyond certain limits of concentration of isotonic NaCl (35 cc. of isotonic NaCl + 15 cc. of sea-water, for example) and of time, the larvae seemed to be injuriously affected by the mixed solution and went down. This occurred both in the light and in the dark. The swimming movement, in this case, was not typical, and the downward movement was probably passive sinking. It was, therefore, hardly due to active geotropism.

From a physiological viewpoint, it is interesting to compare the above observations of the writer with those of Loeb (15, pp. 279–280, 283) and of Ewald (1, pp. 602–603). According to them, negatively heliotropic Polygordius larvae, Copepods, and the nauplii of Balanus perforatus became positively heliotropic on increasing the proportion of NaCl in the mixed solution. And positive animals become "more positive." It seems probable, therefore, that NaCl produces different effects in different species of animals.

a. Returning into Sea-water. The larvae which were thus affected by the excess of NaCl recovered in a few minutes after returning into normal sea-water. Naturally, the larvae which were treated by the less strong solution of NaCl, or by the

TABLE I

Effects of 0.35 m $MgCl_2$ on the geotropism

SEA WATER	$MgCl_2$	IMMEDIATELY AFTER THE TREATMENT	WITHIN 1 MINUTE	1.30-3 MINUTES AFTER THE TREATMENT
50 cc.	0	All show positive heliotropism and negative geotropism.	Practically all at the window side of the top of the graduate in ring.	Practically all at the window sid of the top of the graduate in rin
45 cc.	5 cc.	Practically all show positive heliotropism and negative geotropism.	Majority at the window side of the top in ring and number still increasing there.	Majority at the window side of th top in ring; a very few at the neg tive side of the top, or swimmi about there.
40 cc.	10 cc.	Majority show positive heliotropism and negative geotropism; a very few swimming downward.	Majority at the window side of the top in ring and number still increasing there.	Majority at the window side of th top in ring; a very few at the neg tive side of the top; a very fe at bottom.
35 cc.	15 cc.	Majority show positive heliotropism and negative geotropism; a very few swimming downward.	Many at the window side of the top in ring and number still increasing there.	Many at the window side of the to in ring; a very few swimmi downward; a very few at botto more at the window side.
30 cc.	20 cc.	Many show positive heliotropism and negative geotropism; a very few swimming downward; (some contracted a little, though recovered about 10 seconds later?).	About 40 per cent at the window side of the top in ring and number still increasing there; a very few at the negative side of the top; a few swimming downward.	Many at the window side of the to in ring, but number decreasi fast; a very few at the negati side of the top; considerable nu ber swimming downward; a few bottom.
25 cc.	25 cc.	Some show positive heliotropism and negative geotropism; others positive geotropism; (considerable number contracted, though recovered 10-15 seconds later?).	About 25 per cent at the window side of the top in ring; many swimming about near top; quite a few show beautiful positive geotropism.	Number at the window side of th top decreasing fast; many sho the most beautiful positive ge tropism and downward swimmi typical in every respect; a few bottom.
20 cc.	30 cc.	Some show positive heliotropism and negative geotropism; others positive geotropism; (considerable number contracted, though recovered 10-15 seconds later?).	About 20 per cent or less at the window side of the top; considerable number swimming downward.	At 1.30 minutes after the treatmen a very few at the window side the top; many show beautif positive geotropism (rather slo swimming movement?); quite few at bottom.
15 cc.	35 cc.	Some show positive heliotropism and negative geotropism; others positive geotropism; (many contracted, though recovered 10-15 seconds later?).	About 15 per cent at the window side of the top, though number already decreasing; many (slowly?) swimming downward.	At 2.30 minutes after the treatmen practically none at top; quite few (slowly?) swimming dow ward; the rest at bottom swi ming about.
10 cc.	40 cc.	Some show positive heliotropism and negative geotropism; others positive geotropism; (many contracted, though majority recovered 10-15 seconds later?).	About 10 per cent at the window side of the top, though number already decreasing; many slowly swimming downward (some sinking?).	At 2 minutes after the treatmen practically none at top; a fe slowly swimming (some sinking downward; the rest at botto evenly distributed.
5 cc.	45 cc.	A few show positive heliotropism and negative geotropism; some positive geotropism; (many contracted, though majority recovered 10-15 seconds later?).	Quite a few at the window side of the top; many slowly swimming downward (some sinking?).	At 2 minutes after the treatmen practically none at top; majori at bottom evenly distributed.
0	50 cc.	Many contracted, though majority recovered 10-15 seconds later.	About 20 seconds after the treatment, a few at the window side of the top; majority slowly swimming downward; some sinking.	At 2 minutes after the treatmen all at bottom evenly distribute

TABLE I

e geotropism of Arenicola larvae in daylight

THE TREATMENT	5 MINUTES AFTER THE TREATMENT	10 MINUTES AFTER THE TREATMENT	15 MINUTES AFTER THE TREATMENT	20-30 MINUTES AFTER THE TREATMENT	DEATH AFTER THE TREATMENT
ne window side raduate in ring.	Practically all at the window side of the top of the graduate in ring.	Practically all at the window side of the top of the graduate in ring.	Practically all at the window side of the top of the graduate in ring.	Practically all at the window side of the top of the graduate in ring.	About 25 days after, all dead.
dow side of the few at the nega- p, or swimming	Majority at the window side of the top in ring; a very few at the negative side of top; a very few at bottom.	Majority at the window side of the top in ring; a very few at the negative side of top; a very few at bottom.	Majority at the window side of the top in ring; a very few at the negative side of top; a very few at bottom.	Majority at the window side of the top in ring; a very few at the negative side of top; a very few at bottom.	About 20 days after, all dead.
dow side of the few at the nega- op; a very few	Many at the window side of the top in ring, though number decreasing; a very few at the negative side of the top; a few at bottom.	About 30 per cent at the window side of the top in ring; a few at the negative side of the top; many swimming downward; considerable number at bottom.	A few at the window side of the top in ring; majority at bottom, more at the window side.	Practically none at top; majority at bottom, more at the window side.	About 14 days after, all dead.
side of the top few swimming few at bottom, w side.	About 50 per cent at the window side of the top in ring, though number decreasing; considerable number swimming downward; quite a few at bottom, more at the window side.	None at top; a few swimming downward; the rest at bottom, more at the window side.			About 11 days after, all dead.
side of the top ber decreasing t the negative siderable num- nward; a few at	Practically none at top; a few swimming downward; many at bottom, more at the window side.				About 10 days after, all dead.
ow side of the t; many show l positive geo- vard swimming spect; a few at	Practically none at top; a few swimming downward; many at bottom, more at the window side.				About 9 days after, all dead.
the treatment, window side of how beautiful n (rather slow ent?); quite a	None at top; a few swimming downward at the lower part; majority at bottom evenly distributed.				About 8 days after, all dead.
the treatment, t top; quite a imming down- bottom swim-					About 3 days after, all dead.
the treatment, at top; a few some sinking?) est at bottom					About 25 hours after, all dead.
the treatment, top; majority distributed.					About 14 hours after, all dead.
the treatment, ly distributed.					About 13 hours after, all dead.

stronger solution during a shorter time, recovered more rapidly than those which were treated by the stronger solutions or during a longer time. Although the recovered larvae swarmed at the top, they still remained a while negative to light.

2. The effect of potassium chloride

Potassium chloride has the same effect qualitatively as NaCl. The reversal of the normal positive heliotropism by KCl, was, however, shown by fewer larvae than that by NaCl, probably owing to the stronger toxic action of the former.

Ishikawa (5, p. 20) and Ewald (1, p. 603) seem to have obtained, by the addition of NaCl and KCl to the medium, the same effects on the heliotropism of the nauplii of Balanus perforatus and in general physiology of Amoeba, as did the writer in the case of Arenicola larvae. But Spaeth, on the contrary, has obtained "reciprocal" effects of these two salts on the melanophores of fishes (35, pp. 543–544).

a. Returning into sea-water. The larvae treated by isotonic KCl recovered their normal reactions in sea-water more slowly than those treated by isotonic NaCl. There was, however, no other essential difference between the "after-effects" of KCl and NaCl.

3. The effect of Calcium chloride

If the reversal of the normal negative geotropism obtained on adding hypertonic $CaCl_2$ solution to sea-water was due to the increase in osmotic pressure, no change should be expected on addition of isotonic $CaCl_2$ to sea-water. This, however, was not the case. Even though the larvae remained positive to light in this mixture, they gradually became positive to gravity and swam downward. Their downward swimming was typical in every respect; and their orientation was perfect.

As is well known, pure or nearly pure isotonic $CaCl_2$ solution (e.g., a mixture of 45 cc. of isotonic $CaCl_2$ + 5 cc. of sea-water) is very toxic. The downward swimming of the larvae, therefore, was not so perfect in the more concentrated as in the less concentrated mixtures of this kind. The best results in a series

of ten different mixtures of isotonic $CaCl_2$ solution and sea-water were obtained with equal proportions of isotonic $CaCl_2$ solution and sea-water.

According to Ewald, in experiments on the heliotropism of the nauplii of Balanus, "calcium chloride belongs to the same group as the two first mentioned salts" (1, p. 603), i.e., NaCl and KCl. In the case of Arenicola larvae, on the contrary, $CaCl_2$ had an entirely different action from NaCl and KCl. It is well known through the work of Loeb (17), R. S. Lillie (6–10), Mathews (26), Osterhout (32–33), and others that calcium salts have in general an action physiologically antagonistic to Na and K salts. It is little wonder, therefore, that the larvae in the above $CaCl_2$ solutions behave differently from those in the NaCl or KCl solutions.

a. Returning into sea-water. The larvae, when returned to sea-water, became strikingly negative to light, and a considerable number of individuals remained for a long time in this condition. Their upward swimming was considerably retarded in the light, on account of this negative heliotropism.

Pure isotonic $CaCl_2$ and NaCl are strongly toxic and irreversible changes are rapidly produced.

4. The effect of Magnesium chloride

Through the work of R. S. Lillie (6–10), it is well known that the action of isotonic $MgCl_2$ solution on Arenicola larvae is similar in many ways to that of isotonic $CaCl_2$ solution. It was thus to be expected that isotonic $MgCl_2$ would have the same effect on the reversal of negative geotropism as isotonic $CaCl_2$. This was found to be the case. Moreover, a mixture of 25 cc. of isotonic $MgCl_2$ solution and 25 cc. of sea-water produced a much better result in reversing the normal negative geotropism than the similar mixture of $CaCl_2$ solution. About two minutes after placing the larvae in this mixture, both in darkness and daylight, a majority of the larvae were seen actively swimming downward.

Pure, or nearly pure, isotonic $MgCl_2$ solution is toxic. The larvae all died in the pure solution within thirteen hours.

TABLE II

*Effects of 0.35m $MgCl_2$ on the geotropism of Arenicola larvæ in darkness**

SEA WATER	$MgCl_2$	2-3 MINUTES AFTER THE TREATMENT	5 MINUTES AFTER THE TREATMENT	10 MINUTES AFTER THE TREATMENT	15-20 MINUTES AFTER THE TREATMENT	25-30 MINUTES AFTER THE TREATMENT
50cc.	0	All at top.	All at top.	All at top.	All at top.	All at top.
45cc.	5cc.	All at top.	Practically all at top; only 2 or 3 at bottom.	Majority at top; a very few at bottom.	Majority at top; a very few at bottom.	Majority at top; a very few at bottom.
40cc.	10cc.	Practically all at top; a very few at bottom.	Majority at top, or near top; a very few at bottom.	About 30 or 40 per cent at top; many swimming downward; considerable number at bottom.	About 80 per cent at bottom.	Majority at bottom.
35cc.	15cc.	Considerable number at top; quite a few swimming downward; about 60 per cent at bottom.	A few at top; considerable swimming downward; many at bottom.	None at top; a very few swimming downward; the rest at bottom.		
30cc.	20cc.	Practically none at top; considerable number swimming downward; the rest at bottom.				

25cc.	25cc.	Practically none at top; considerable number swimming downward; the rest at bottom.
20cc.	30cc.	None at top; a few swimming downward at the lower part.
15cc.	35cc.	None at top; a few (slowly?) swimming downward at the lower part.
10cc.	40cc.	All at bottom.
5cc.	45cc.	All at bottom, at two minutes after the treatment.
0	50cc.	All at bottom, at two minutes after the treatment.

* Observations were made as quick as possible at intervals indicated in the table.

It was not the writer's intention to discuss heliotropism in the larvae. Therefore, he did not study in detail the effects of these solutions on heliotropism. According to Mast (24, p. 282), however, the larvae became negatively heliotropic in the mixtures of $MgCl_2$ and sea-water. The writer also found this to be true, at least in some individual cases.

The following tables give a detailed account of the observations made in different mixtures of $MgCl_2$ solution and sea-water, both in the light and in the dark.

a. Returning into sea-water. Generally speaking, the larvae treated by mixtures of sea-water and $MgCl_2$ solution recovered more rapidly after returning in sea-water than did those which were treated by similar $CaCl_2$ mixtures. The larvae, also, became negatively heliotropic, after returning into sea-water.

5. The effect of Magnesium Sulphate

In attempting to make up a $MgSO_4$ solution isotonic with sea-water, a mistake in calculation was made by the writer, 0.47 m. being used instead of 0.9 m. Since the result in using 0.47 m. $MgSO_4$ was closely similar to those of isotonic $MgCl_2$, it is apparent that the difference in osmotic pressure had little practical consequence. In their qualitative effects the two salts are alike.

6. Antagonistic effect of Sodium and Calcium Chlorides

Antagonism between sodium and calcium is well known. As already pointed out, the larvae in the sea-water mixed with isotonic NaCl solution became negative to light, but remained negative, as previously, to gravity. On the other hand, the larvae in the sea-water mixed with isotonic $CaCl_2$ solution became positive to gravity, and remained positive, also, to light. If, therefore, antagonism exists between the two salts, the negativating effect of sodium on heliotropism should be antagonized by addition of $CaCl_2$, and, on the other hand, the positivating effect of $CaCl_2$ on geotropism should be antagonized by addition of NaCl. This was the case, as expected. In the mixture of 30 cc. of isotonic NaCl and 5 cc. of isotonic $CaCl_2$ plus 20 cc. of sea-water, negative heliotropism was produced to a much less degree than in the

mixture of 30 cc. of isotonic NaCl and 20 cc. of sea-water. There was not, however, relatively so great a decrease of positive geotropism in the mixture of 25 cc. of isotonic $CaCl_2$ and 10 cc. of isotonic NaCl plus 25 cc. of sea-water, as compared with the mixture of 25 cc. of isotonic $CaCl_2$ plus 25 cc. of sea-water without additional NaCl, although there was a definite retardation.

A similar experiment was tried with isotonic $MgCl_2$ solution, and practically the same results were obtained as the above, though a little less marked (?).

7. Effects of anaesthetics

According to R. S. Lillie (7, 9–10), the effects of isotonic $CaCl_2$ or $MgCl_2$ resemble in many respects those of anaesthetics. It might be expected, therefore, that the larvae in the mixture of 1.5 cc. of 95 per cent alcohol with 50 cc. of sea-water, or 0.3 cc. of ether with 100 cc. of sea-water (which was the optimum amount of these substances for the animals used), should become positive to gravity. This was tried by the writer without success. The tests, however, were not very extensive.

8. Effects of other chemicals

Besides the salts and anaesthetics above mentioned, $\frac{N}{20}$ KOH, $\frac{N}{20}$ NaOH, $\frac{N}{20}$ NH_4OH, CO_2, $\frac{N}{20}$ HCl, and $\frac{N}{20}$ H_2SO_4 were tested in optimum concentrations to determine whether they have any effect on normal geotropism of the larvae. No definite results, however, were obtained. Some of these chemicals—acids and NH_4OH, for example—were noticed to produce a negativating effect to light.[2] But, as Mast rightly pointed out, "in no instance was the negative reaction as marked and precise as the positive had been" (24, p. 282).

[2] As already stated, it was not the writer's intention to study heliotropism in the larvae. And the method was not well adapted for this purpose. He takes the liberty here, however, to quote fully Professor R. S. Lillie's comments on the writer's manuscript as follows:

"A large number of substances and changes of condition produce a tempotary reversal of the sense of phototaxis in these larvae.

"Besides the instances cited in the footnote of my paper (7, 1909, P. 35), I have found the larvae to become negative, to a greater or less degree, after treatment

IV. DISCUSSION

From his observations on heliotropism of Arenicola larvae and also others, Mast concludes that "the effect of the different chemicals is not specific. The chemicals appear to produce changes in the general state of the organism as a whole or a unit" (24, pp. 279–280). As far as the writer's observations on geotropism of the larvae are concerned, Mast's conclusion does not seem quite right. Magnesium and calcium chlorides had a positivating effect; sodium and potassium chlorides on the other hand, did not. Acids, alkalis, and narcotics, though experiments with these chemicals were not extensive, did not reverse the sense of geotropism in the larvae.

According to R. S. Lillie, "$MgCl_2$ and similarly acting solutions (including $CaCl_2$) appear to *decrease* the permeability of the tissues, and so prevent the ionic transfer on which stimulation depends. The general action of anaesthetics consists in *decreasing the normal permeability;* stimulating agencies, (NaCl and KCl, for example), on the other hand, have the reverse effect." "The chemical effect of the above changes in permeability depends essentially on their influence in varying the rate at which carbon dioxide leaves the cell. The velocity of the oxidative energy-yielding processes whose end product is CO_2 is thus varied with the rate of removal of this latter substance from the system; the velocity is accordingly increased during the increased permeability of stimulation, and is decreased during anesthesia or inhibition" (7, p. 44).

This hypothesis is rather interesting to note, because it would suggest that the reversal of negative geotropism by means of $MgCl_2$ or $CaCl_2$ may be due to the decrease or loss of the sense of geotropism (or geotropic response) in the larvae. In other words they may become "passive" on addition of the salts to

with appropriate solutions of the following substances: tannic, phosphotungstic, phosphomolybdic and picric acids; ethyl alcohol, ethyl ether, benzol, toluol, xylol, methyl and ethyl acetates, ethyl butyrate, acetic and sulphuric acids, sodium hydrate, saponin, nicotine, blood serum of skate. These observations were made in the summer of 1909. After exposure to the solutions of these substances for a few minutes, many larvae—in some cases the majority—become negative immediately after returning to normal sea-water. Later they become again positive. The conditions of this change require further investigation."

sea-water. The anterior end of the larvae is heavier than the posterior[3] and there is little doubt that orientation of the larvae against gravity is in normal sea-water due essentially to "protoplasmic materials of different specific gravity," as Lyon thinks in Paramecium (21, p. 429). If so, in sea-water containing an excess of $MgCl_2$ which acts as an anaesthetizing reagent, the larvae may be supposed entirely to lose the sense of stresses or pulls of protoplasmic materials of different specific gravity which occur when the organisms are in one position with respect to the vertical, and become "passive" to the pull of gravity.

A difficulty in this view, however, arises from the fact that the downward swimming of the larvae in the mixture of isotonic $MgCl_2$ or $CaCl_2$ and sea-water is typical in every respect. No abnormality of ciliary movements is noticeable, at least in the solution of 25 cc. of isotonic $MaCl_2$ or $CaCl_2$ and 25 cc. of sea-water. In other words they show no signs of ciliary anaesthesia. Another objection is that the optimum amount of alcohol or ether, that is, 1.5 cc. of alcohol in 50 cc. of sea-water, or 0.3 cc. of ether in 100 cc. of sea-water, does not produce any definite positivating effect, as $MgCl_2$ or $CaCl_2$ does. The reaching of a satisfactory theory of these effects, therefore, belongs to the future.

V. SUMMARY AND CONCLUSION

1. Addition of isotonic NaCl solution to sea-water has no reversing effect on the normal negative geotropism of the larvae of Arenicola cristata. It has, however, a negativating effect on the normal positive heliotropism, of the larvae, though not marked.

2. Returning into sea-water after the treatment, the larvae recovered from the toxic effect of NaCl and swarmed at the top again, remaining, however, negative to the light a while.

3. Addition of isotonic KCl solution to sea-water has essentially the same effects as those of NaCl just mentioned.

4. The toxic action of isotonic mixtures of KCl and sea-water is much stronger than that of NaCl mixtures.

5. In a mixture of isotonic $CaCl_2$ solution and sea-water, the

[3] On this matter, a separate paper will be prepared by the writer.

normal negative geotropism of the larvae is reversed. The best result is obtained in a mixture of one-half of isotonic $CaCl_2$ solution and one-half of sea-water. These solutions do not reverse the normal positive heliotropism.

6. Pure isotonic $CaCl_2$ solution is more toxic than pure isotonic NaCl solution.

7. The larvae, when returned to sea-water after treatment in mixtures of sea-water and isotonic $CaCl_2$ solution, become strongly negative to light.

8. A mixture of isotonic $MaCl_2$ solution and sea-water has effects similar to those of equivalent mixtures of $CaCl_2$. In mixtures of equal parts of isotonic $MgCl_2$ solution and sea-water, however, the reversal of negative geotropism is more pronounced. Magnesium chloride, also, seems to have some negativating effect to light, at least for some individuals.

9. The $MgSO_4$ solution, which was used by the writer, though not isotonic with sea-water, was found to produce effects similar to those of isotonic $MgCl_2$, though not so marked and precise.

10. Addition of a small amount, say, 4 or 5 cc. of isotonic $CaCl_2$ or isotonic $MgCl_2$, especially the former, to the mixture of isotonic NaCl and sea-water, say, 30 cc. of the former in 20 cc. of the latter, antagonizes the action of the NaCl, preventing the reversal of heliotropism by the latter salt.

11. Addition of the optimum amount of alcohol or ether to sea-water produces no definite reversal of negative geotropism like that produced by isotonic $MgCl_2$.

12. Addition of the optimum amount of $\frac{N}{20}$ KOH, NaOH, NH_4OH, CO_2, HCl, or H_2SO_4 produces no reversal of negative geotropism, although some chemicals, acids and NH_4OH, for example, have a reversing effect on positive heliotropism.

13. Since the magnesium or calcium chloride solutions which were used are isotonic with sea-water, the reversal of negative geotropism of the larvae is not an osmotic effect. And since the action of $MgCl_2$ or $CaCl_2$ is different from that of NaCl or KCl, the reversal effect of the former two salts seems to be specific. The chlorine ions in these four salts being the same, the specific action of isotonic $MgCl_2$ or $CaCl_2$ seems therefore to be due essentially to Mg^{++} or Ca^{++}–ions.

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FUNCTIONAL VARIATIONS IN CONTRACTIONS OF DIFFERENT PARTS OF THE SMALL INTESTINE

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In the course of some studies upon segments of rabbit's intestine contracting rhythmically in warm oxygenated Ringer's solution, after the method used by Magnus (1) Hoskins (2) and others, it became necessary to observe two or more pieces under identical conditions—that is—they had to be beating together in the same tube with the same temperature, oxygen supply, etc. As soon as two or three pieces from different parts of the small intestine were brought together in this way it was plainly to be seen that the rhythm of the upper end of the small bowel is almost double that of the lower (see fig. 1). At Dr. Cannon's suggestion the original problem was dropped for a time and attention was concentrated on this matter of rhythm.

TECHNIC

Rabbits are particularly adapted for the work and have been used exclusively so far. The rhythm is rapid and regular; the segments can be used in their entirety without the trauma attendant upon separating the muscle layers or cutting strips; the duodenal loop is unusually long and accessible; and in this animal the longitudinal muscle is especially active.

The animals were kept under urethane (2 grams, by stomach, per kilo of body weight), and segments were removed from time to time as needed, the abdomen being closed with clips. Anesthesia should be brought about as quietly as possible, because if the animal has been frightened the cecum is likely to be baggy and full of gas; the intestinal tone poor and the segments often

unreliable. Older rabbits are better in this regard as they seem less disturbed by the preliminary handling. Animals with diarrhoea, as evidenced by the soiled tail, should be avoided. The only effect of urethane on the segments was a slight drop in tone appearing with a dilution of one to four hundred, so that it could hardly be a disturbing factor. Hoskins (3) also thinks it has little effect on the intestine.

In removing the segments it was found best to begin at the pylorus and to proceed downward, as the bowel soon became dis-

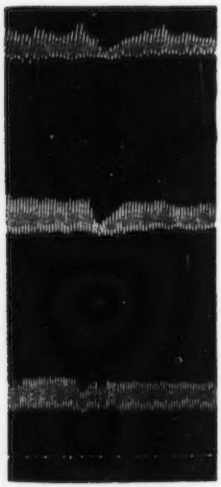

Fig. 1

Fig. 2

Fig. 1. From above downward: lower ileum, upper ileum, and duodenum. The duodenum recovers first from the depression caused by 1:55,000,000 adrenalin. Time marking in all the tracings shows 30 second intervals.

Fig. 2. "U" loop of duodenum showing the irregularity and poor amplitude after stasis.

tended and atonic after ligation of the ileum. When this happened segments from the duodenum did not beat well—the amplitude was poor, the rhythm was irregular, and fatigue appeared early (see fig. 2). Such effects were much less noticeable in segments of ileum and jejunum, and further work must be done to see if these

changes in the duodenum are due simply to a greater vulnerability of this region or to a local action of the toxins described by Whipple, (4) Bunting and Jones (5) and others. It was easily proved that the sequence of removal of the segments from different parts has no effect on the rhythm.

Trauma in the jejunum and ileum was found to have little effect on the segments except to cause a rapid loss of tone at first; such pieces last well and the rhythm is unchanged. The greatest care must be taken, however, if good tracings are to be obtained from the duodenum. The main vessels should be clamped and the segment removed quickly without pulling or handling. Any hemorrhage can then be attended to. Another sign of the greater vulnerability of the duodenal segments is their slow recovery after the shock of transferrence to the beaker. It may be ten minutes before they are beating well, while segments from other parts of the gut may not miss a beat from the time they are cut until they are attached to the lever. This is of interest in view of the fact commonly observed by abdominal surgeons that in operations on the intestine, "The nearer one approaches the pylorus, other factors being equal, the greater the shock (6) (7)."

The mesenteric side should not be used as it seems to be less sensitive, and it cannot relax properly on account of the adherent connective tissue. Apparently the size of the rabbit makes no difference. Most of those used in this work weighed from 1.7 to 2.0 kilos.

Sudden changes of temperature, however small, must be avoided. Not only must everything that is to be used near the segments be kept in the same water bath at 37°, but frequent readings must be taken in the small tank with a delicate thermometer, especially while changing the fluid or adding substances to be tested. The work of the first few weeks was lost because it had been taken for granted that the temperature in the bath and the small tank would be practically the same. It was found that differences in level in the two fluids, thick glass walls, the bubbling of the cold air, etc., could make a difference of 1–2°C. Hoskins (8) and others have remarked upon the rise which occurs when Ringer's solution containing a trace of adrenalin is washed out.

This is probably due to a slight cooling of the washing fluid in the syringe, as a *drop* in tone can be obtained by having the new fluid .3–.4° warmer. Ringer's solution for washing can be kept in the bath under air pressure, so that on releasing a clamp it will flow through a fine tube reaching to the bottom of the tank. In this way the fluid can be changed without any disturbance; and the test of a man's technic should be his ability to wash a strip without changing its tone. These segments of intestine are really delicate thermometers, sensitive to tenths of a degree, and some of the work done with them in the past may have to be repeated with proper temperature control.

Changes in the rate of oxygen flow affected the tone of the segments, and the compressed air of the laboratory was found more satisfactory on account of its constant pressure. An extra valve was added so that after being adjusted to a convenient flow it did not have to be changed for weeks. A small coil was put in to warm the air as it passed through the bath.

Although it was found that the length of the segment had little effect on its rhythm, a standard of 3.5 cm. was adhered to throughout the work.

RHYTHM

The figures plotted in figure 3 were obtained from the records of the last week's work when the technic was at its best. Twenty-five segments from 9 rabbits were taken and the rhythm noted for 3 minute intervals as soon as the records became steady—usually in the first 15 minutes. Three minute intervals were counted throughout the work to insure greater accuracy.

Temperature changes had next to be taken into account. According to Magnus' (9) table, a strip beating, for instance, 7.5 times per minute at 32°C. will beat 18 times per minute at 42°C. or 2.4 times faster. This coefficient corresponds closely to that obtained for the heart-beat; which, again, has been shown to agree pretty well with that of Arrhenius for the velocity of chemical reaction (10). Hence, in plotting figure 3 I have estimated what each rhythm would be at 37°C. assuming that the rate changes, as in a chemical reaction, 10 per cent for each degree.

As all but four of the readings were taken at temperatures ranging between 37° and 38° only slight alterations had to be made.[1]

It will be seen that after a rapid drop from 17.6 to 15, the line is almost straight to about 10. Hence it may be stated roughly that the rhythm of the small intestine (in the rabbit) varies inversely as the distance from the pylorus. This law holds so firmly that if two segments be taken 20 cm. apart anywhere in the bowel, the upper piece can generally be distinguished by its faster rhythm.

Again, when segments from the jejunum, duodenum or upper

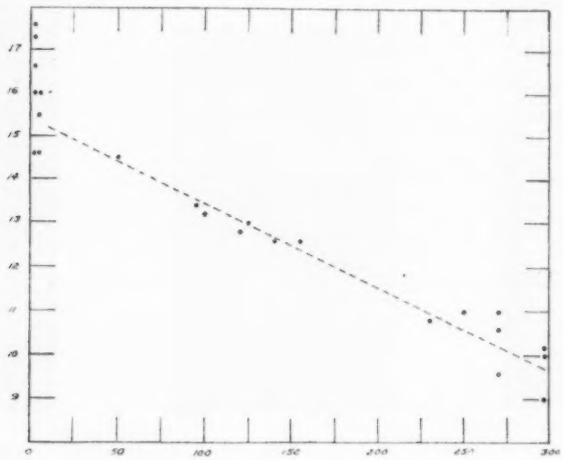

Fig. 3. Rates of rhythm in different parts of the small intestine of the rabbit. Ordinates are rates per minute and abscissae are distances in centimeters from the pylorus. Temperature 37°C.

ileum, 10 to 15 cm. long, were suspended in the tank with levers writing from both ends, and serrefines attached to the mesentery so that the arms of the "U" were 3.5 cm. long, it was found that, as a rule, the oral end kept beating a little faster than the aboral. In "U" strips from the lower third of the bowel, strange to say,

[1] In the chart the small intestine is assumed to be 300 cm. long as those that were measured in situ did not vary much from this average.

the aboral end regularly beat faster. The possible significance of this finding will be seen later.

The oral end of a piece of duodenum with good conductivity can also be distinguished from the aboral end by the greater regularity of its rhythm and amplitude (see fig. 4). This may be due to the fact that it is not subject to the descending impulses which so greatly disturb the lower end. The conductivity seems to be poorer in the rest of the tract as this difference is less fre-

Fig. 4

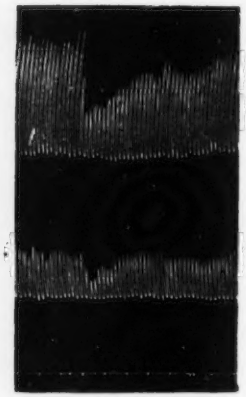

Fig. 5

Fig. 6

Fig. 4. Short piece of ileum allowing but 2.5 cm. between the arms of the "U." The aboral end (upper record) beats irregularly.

Fig. 5. Long "U" segment from upper ileum. Shows the more marked effect of adrenalin on the aboral end (upper record).

Fig. 6. "U" segment from terminal ileum. Shows the prolonged effect of a pinch on the aboral end (upper record).

quently seen in pieces from jejunum and ileum. The problem is complicated by the fact that this function seems to vary quite markedly in the different rabbits used.

Bottazzi (11) states that a regular rhythm in segments of esophagus is a sign of poor conductivity. This function becomes

impaired early in the life of the segment and long-distance pinch reactions are generally best shown in the first half-hour.

ADRENALIN EFFECTS

Another feature distinguishing upper and lower parts of a loop of intestine is the difference in the reaction to adrenalin. The drop is always greater at the lower end; and if the dose be large enough to cause complete stoppage, the upper end will begin beating first. A pinch applied in the middle of the "U" loop shows similarly a greater effect upon the lower end (see figs. 5 and 6). It would indicate either an easier path for depressive stimuli down the bowel or the presence of a constant stream of depression to which the new stimulus has been added. The presence of such a stream is suggested in figure 7 where a sudden increase in the amplitude of the oral end (when it recovered from the shock of attachment to the levers) was associated with a marked drop in the tone and amplitude of the aboral end.

A difference in the reaction to adrenalin is found also on comparing separate strips. The threshold is apparently the same, but the rhythm of the duodenum is more pertinacious and harder to upset. Fig. 1 shows how 1:55,000,000 adrenalin interfered with the rhythm of the ileum for 13 beats, of the jejunum for 11 beats, while the duodenum recovered after 8 beats. This greater steadiness of the duodenum was noted in many tracings.

AMPLITUDE

Tracings from duodenum and ileum generally show enough differences to be distinguished at a glance. Under the most favorable circumstances, the amplitude of the duodenal contractions is rarely over 3 cm. (magnification 20.5:12) while that of the ileum in the last 25 cm. will often be 10 to 12 cm. (see fig. 8). (The segments, be it remembered, are all of the same length.) That this difference in amplitude is not sufficient to account for the difference in rhythm is shown by the fact that the rate may be the same 2–3 hours later when the amplitude, through fatigue, has dropped to 1–2 cm.

SACCULUS ROTUNDUS

It was soon noticed that tracings from the first part of the duodenum and from the terminal ileum could easily be distinguished by the presence of superimposed rhythms. Even after the most careful handling, some segments of duodenum could not be made to beat well. No one rhythm could dominate and the amplitude remained very small (see fig. 9). It was found that all such pieces were from the first 4 cm. of the gut, and the worst

Fig. 7

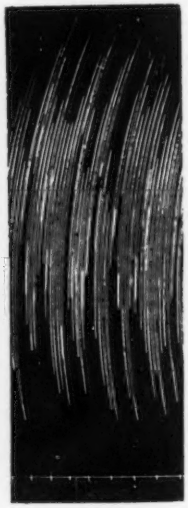

Fig. 8

Fig. 7. "U" segment from the upper duodenum. The spontaneous increase in activity orally (lower record) is associated with a marked drop in tone at the other end of the same short segment.

Fig. 8. Terminal ileum. Wide amplitude with irregularities due to the sacculus.

pieces included a little of the pyloric ring. As strips from the antrum of the stomach could not be made to beat, this interesting subject was dropped for a time.

The superimposed rhythm in the ileum was easier to account for. At the end of the bowel where it enters the cecum there is

a small enlargement about the size and shape of the ball of the thumb. It is called the sacculus rotundus, or the lymphoid sac, but few suggestions have been made as to its function.

This sac was removed with a strip of ileum and suspended in the tank by one hook at the ileo-saccular junction so that the ileum and sac would register on the same drum. It was found that the rhythm of the two was the same—about 10.6 per minute —until they were separated by a snip of the scissors, when the rate of the ileum dropped to 6.6 for a few minutes, while that of the sacculus jumped to 12 (see fig. 10). The ileum had apparently been receiving its rhythm from the sac and it took a few

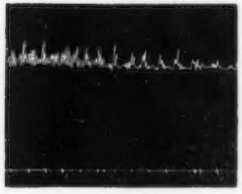

Fig. 9

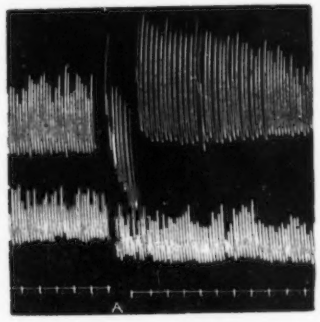

Fig. 10

Fig. 9. Duodenum next to the pylorus. Shows typical irregularities and the narrow amplitude.

Fig. 10. Upper record is of the terminal ileum, lower: of the sacculus. When separated at "A," the tone of the ileum fell so rapidly that the tambour had to be raised. The rate fell temporarily in the ileum and rose slightly in the sacculus.

minutes to develop its own after being isolated. Figure 11 shows an ileum which was so dependent upon the sacculus for its rhythm that it remained almost paralyzed when cut away. That this was not due to traumatism was shown repeatedly. Figure 12 shows how slightly a strip of ileum reacts to being cut across.

RHYTHM IN THE INTACT ANIMAL

On turning to the animal opened under physiologic salt solution, I found very similar conditions. (Anesthesia was the same as before and the spinal cord was destroyed as far up as the interscapular region.) The movements of the duodenum, again, are short and quick, while those of the ileum are long, slow pulls reaching 5 to 10 cm. along the gut. The rhythm in different parts, counted with a stop-watch, closely follows that of the isolated segments. Again, I found that the upper duodenal region has

Fig. 11

Fig. 12

Fig. 11. Upper record is of the sacculus; lower: of the ileum. The ileum has failed to recover after separation from the sac.

Fig. 12. "U" segment from the lower ileum. The only effect of cutting it in two was a slight rise aborally (upper record).

the steadiest rhythm, often beating for hours at a very constant rate. This varies between 17.5 and 21 per minute in different rabbits, much as the pulse in one man holds about 65 while in another it is about 80. The lower ileum is often perfectly quiet for long periods of time. When active its rate varies between 5 and 15 per minute—oftenest 10 to 12. The great difficulty in studying rhythms in the intact intestine is that they change so

markedly with the functional activity of the bowel. For instance, a duodenum which had been running 17.5, 17.5, 18.5, 17.5 dropped to 16 when its contents went on to the jejunum, which had been beating 11 to 12.5. This now took on the rate of 14. A piece of ileum had been 8, 8, 8, 8, for several minutes until a rush wave passed. It was then found to be 10, and twenty minutes later it was back to 9 which seemed its normal rhythm. Another segment of ileum beating 11.5, 11, dropped to 10 as a slow rush started high up in the bowel. Just before the wave arrived it was at 6, and it returned to 10 soon after. Further work is now being done on this phase of the subject.

The sacculus generally lies inactive unless the intestinal contents start to go through the ileum pretty rapidly. When it is active it can be plainly seen setting the pace so that peristalsis of the last few centimeters of the ileum is reversed. The more rapid rhythm noted in the aboral end of an excised segment of ileum is probably another sign of the tendency to delay in this region. That stagnation does occur in the rabbit is certain, as the dark feces can easily be seen through the thin walls of this part of the gut. Bunting and Jones (12) found in these animals that, although a ligature anywhere in the upper half of the bowel caused death in 15 to 48 hours, if the obstruction were placed close to the cecum, life might be prolonged for a week. The ileum, then, seems to tolerate stagnation almost as well as does the cecum, because the inclusion of this organ in the area of stasis by tying on the colonic side prolongs life only four days more. Hertz (13) states that in man also the food remains in the terminal ileum and "undergoes digestion actually for a greater period than in the stomach." It is well known clinically that inflammation of the cecal region—most commonly appendicitis—will greatly increase this stasis.

TONE

The difference in amplitude noticed in the tracings would indicate differences in tone. A segment of duodenum taken from a young rabbit that had been much frightened, and whose intestine was flabby and full of gas, beat with almost as good amplitude as

does a segment of ileum. Bottazzi (14) showed on the esophagus of the toad that as he raised the tone with feeble faradic stimulation, the amplitude of the contractions diminished and the rate increased. The same thing can be seen with the spontaneous tonus waves of the colon (see fig. 13). Woodworth (15) noticed in the frog's stomach that as the tone dropped the amplitude rose, and such instances could be multiplied from the literature.

A similar difference in tone is seen in the cat. It is almost as hard to attach a tiny wire serrefine to the duodenum of a cat as it is to pick up the wall of the gastric antrum or of the colon—the surface is so tense and resilient. There is no such difficulty in the ileum where a little fold can be easily pinched up.

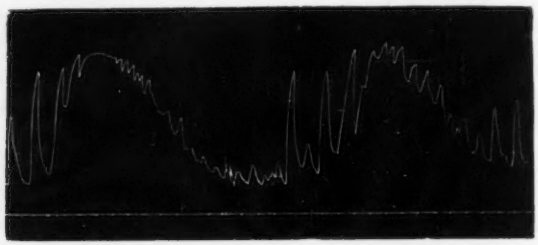

Fig. 13. Segment of rabbit's colon showing the normal tonus waves with small contractions superimposed. Time in half-minutes.

The more rapid rhythm associated with increased activity of the bowel is probably a corrollary of the increased local tone which Cannon (16) has shown to be so essential to the maintenance of the segmenting movements. I have placed two serrefines 3.5 cm. apart on an active and apparently stretched piece of rabbit's duodenum and have found them 8 cm. apart later when this region had emptied and quieted down.

IRRITABILITY

It could easily be shown in the rabbit opened under salt solution that light pinches of approximately equal strength have a greater, more rapid and more constant effect on the duodenum than on any

other part of the tract. Later, while studying segments of intestine, it was noticed that the threshold for thermal stimuli is distinctly lower in the duodenum. Sudden, small changes in temperature incident to changing the Ringer's solution had a distinct effect on the tone of the duodenum, while they were subliminal for the jejunum and ileum. Figure 14 shows such a drop in tone due to the rise of 0.2°C. in the solution. Bayliss and Starling (17) believed that the "higher excitability of the duodenal end of the gut" might have something to do with the preponderance of the descending contractions in the normal animal.

Fig. 14. From above downward: lower ileum, upper ileum and duodenum. A rise in temperature of .2°C. caused a drop in the base-line of the duodenal tracing only.

CONCLUSION

Most investigators who have used the excised segments of intestine have been so much interested in pharmacologic problems that they not only paid no attention to rhythm, but most of them failed even to note whence they cut their pieces—from duodenum, jejunum or ileum. Even Magnus, who has done most of the

purely physiologic work on the segments, seems to regard the small intestine as a tube of uniform properties throughout.

Cannon (18) noticed with the X-ray that peristalsis in the first part of the duodenum is much more rapid than normal peristalsis elsewhere in the small intestine. He says, "The masses once started go flying along, turning curves, whisking hither and thither in the loop, moving swiftly and continuously forward. If this process is true also for man, the region beyond the duodenum would naturally be 'jejune.'"

This brings us to the question: what influence have these differences in rhythm, tone and irritability upon the onward progress of chyme through the tract? In the heart-tube, "the rhythmic power of each segment varies inversely as does the distance from the sinus"(19) and this difference is so important that the current is reversed if the aortic end be made to beat faster (20). Bottazzi (21) ascribed the aboral course of the waves in the esophagus of Aplysia Limacina to the greater tone and irritability of the oral region in which they arose; and Cannon (22) in comparing the tonus-ring to the sinus venosus has described the mechanism of the reverse waves of the colon in terms well known in heart physiology.

Great caution must be exercised if any such comparison is to be made in the *small* intestine because there the progress of contents so little resembles that of blood through the heart. The swaying movements in the rabbit or the segmenting contractions in the cat are not progressive in character. In the rabbit, chyme is churned to and fro in a loop of bowel for an hour or more, the two ends of the loop being in a way antagonistic. From time to time powerful contractions take place and a peristaltic rush seems imminent; but often the two ends appear to be evenly matched and the bowel quiets down again. Finally the balance is upset and a peristaltic rush moves the material onward to another loop. Other things being equal, it seems reasonable to suppose that if the oral end of a loop sends off 16 waves to the aboral end's 15, the balance will be upset ultimately in its favor and the rush will proceed aborally. If the oral end have, moreover, greater tone, greater irritability and a better developed musculature, there

would be still less chance for anastalsis. It seems also more than a coincidence that in the duodenum and jejunum, where the muscle is sensitive and the rhythm active, the progress of the contents should be rapid, while in the ileum, where the rhythm is slower, there should be considerable stagnation.

It is interesting to note that when Lucas (23) obtained simultaneous records from cannulae in different parts of the ureter, he made the "surprising observation that each represented a radically different curve." The waves in the middle were large—4 to 6 per minute; while those from the renal pelvis were small—20 to 60 per minute; and those in the upper part of the ureter were less rapid than in the pelvis. He obtained only two records from the lower end and these were conflicting.

Further work must be done on other animals and on the subjects of tone, irritability and conductivity just touched upon in this paper.[2] Interesting points may be brought out by histologists and embryologists. For instance, Gerlach (24) has shown that the thickness and complexity of Auerbach's plexus varies markedly in different parts of the tract. In the fundus of the stomach (of guinea pigs), the meshes are irregular, about 300μ in diameter. The number of ganglion cells increases towards the pylorus, and they form small ganglia which approach each other more and more. Two to three centimeters from the pylorus the fibers become much thickened, they are studded with ganglion cells, and the mesh is close. This picture is found also in the first 5–6 cm. of the duodenum. Below this the fibers gradually lose their cells and the mesh becomes wider—450 x 180μ. In the cecum the measurements are 1200 x 480μ.

He remarks upon the greater development of the muscle in the antrum of the stomach and the first part of the duodenum and the gradual thinning out down the intestine. The thickness of the nervous plexus from stomach to anus varies directly as the thickness of the muscle layers. That the rhythm and size of peristaltic waves may depend upon the structure of the nerve-

[2]Recently in the Surgical Research Laboratory of the University of California I have observed similar gradations in rhythm in the small intestine of the cat and dog, opened under warm salt solution.

net has been shown by Biedermann in his studies on the foot of the land snail (25). Mall (26) has shown that in early fetal life the duodenum is much larger than the rest of the small intestine; so large, in fact, that it would be taken for a part of the stomach were it not for the position of the ducts.

With pleasure do I express my sense of indebtedness to Dr. Walter B. Cannon for many suggestions and criticisms, and for his having extended to me the privileges of the Harvard laboratory.

SUMMARY

Segments of the rabbit's small intestine, beating in warm oxygenated Ringer's solution, have a much (about 50 per cent) more rapid rhythm when taken from the duodenum than when taken from the lower ileum. Roughly expressed, the rate varies inversely as the distance from the pylorus.

These differences in rate observed in isolated segments have been found true also of the two ends of the small intestine as seen in an animal opened under warm salt solution.

The oral end of an intestinal loop beats with more regular rhythm and amplitude than the aboral end.

The upper end of a loop, or a segment from the upper intestine, is less affected by adrenalin and recovers more rapidly from the inhibitory influence, than a lower region.

The amplitude of contraction of segments with approximately the same length is greater with segments from the ileum than with those from the duodenum.

The rhythm of a segment of the terminal ileum seems to be determined by the rapid rhythm of the sacculus rotundus, for when the two are separated, the segment from the ileum beats at a slower rate.

The more rapid rhythm and the smaller amplitude of duodenal segments are apparently associated with a greater tone of this region compared with the lower ileum.

The duodenal region is more irritable to mechanical and thermal stimulation than lower parts of the small intestine.

It is suggested that the greater tone and irritability and the faster rhythm of the upper part of the small intestine are associated with more rapid peristalsis in this region.

San Francisco.

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THE INFLUENCE OF PITUITRIN ON RESPIRATION

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With the rapid development of the physiology of the internal secretory organs, a knowledge of the influence they exert on the respiratory mechanism seems desirable. In the hope of throwing light on this subject a series of investigations of which this is the second are being undertaken in this laboratory.

So far as we have been able to find, no work has been undertaken to study the character of the respiratory responses as a result of introducing pituitary extract into the circulatory system. Houghton and Merrill found that the rate of the respiratory movements are increased in number after injecting pituitrin.[1] Paukow on the other hand found that the respiratory movements are made shallow or stopped entirely in the rabbit when large doses of pituitrin are given intravenously.[2] These results were recorded, however, by means of a pneumograph, which is too insensitive to show the character of the effects on respiration.[3]

The animals used in our investigations were chiefly urethanized cats. Some observations were also made on urethanized dogs. In recording respiratory movements it is essential to have a uniform anaesthetic. Otherwise the respiratory movements will be irregular and the interpretation of the results difficult.

The method of recording the respiratory movements and of introducing the pituitary extract were the same as used by us in a preceding investigation on the influence of adrenalin on

[1] Houghton and Merrill: Jour. Am. Med. Assoc., 1908, 51, p. 1849.
[2] Nice: this journal. 1914, xxxiii, p. 204.
[3] Paukow: Pfluger's Archiv f.d. gesampte physiologie, 1912, 147 s89.

respiration.[4] A median incision was made through the abdominal wall and an S-shaped hook attached to the diaphragm about midway between the central tendon and the lateral chest wall. From the S-shaped hook a thread was passed over a pulley and attached to a lever which recorded the movements of the diaphragm on a revolving drum. The up-strokes represent expiration and the down-strokes inspiration.

To study the relation of the effects of pituitrin on respiration and compare it with that on the circulatory system, in some experiments the blood pressure was recorded from a femoral artery by means of a mercury manometer.

The pituitrin used was obtained from Parke, Davis & Co. and was fresh. It was put up in one centimeter ampoules. The strength of solution injected was chiefly a 1:25 made by diluting the stock solution with distilled water. In some of the experiments a 1:100, 1:50, 1:10, and even the full strength of the stock solution was used. At first the extract was diluted with normal salt solution. It was found, however, that diluting with distilled water gave the same results as diluting with salt solution, so distilled water was used in all succeeding experiments.

The pituitary extract was injected at a uniform rate into an external jugular vein deep down in the neck by means of a graduated syringe.[5]

The experimental procedure was as follows. The record of the respiratory movements was observed for a time as they were written on the drum. When the nature of the curve had been obtained, 0.3 cc. of pituitary extract was introduced into the circulatory system through a cannula inserted into an external jugular vein. A key was closed to show when the solution was being given. Then an interval of several minutes was allowed in order to let the effects of the pituitrin pass off. Then a second dosage, usually 0.6 cc. was given. After the effects of this dosage had passed off, 0.9 cc., 1.2 cc., and 1.5 cc. were introduced. This graduated increase of dosage was usually

[4] Nice, Rock, and Courtright: this journal, 1914, xxxiv, p. 326.

[5] See Cannon and Lyman: this journal, 1913, xxxi, p. 376; Also Nice, Rock, and Courtright, loc. cit.

followed but not always. In a few cases there was little or no response to the injection of 0.3 or 0.6 cc. of a 1:25 pituitary extract; we accordingly increased the doses at once to 1.2 cc. of 1:25 solution, or 0.3 cc. of a 1:10 solution.

The dosages were increased at a uniform rate to find whether there is a direct relation of the respiratory responses to the amount of pituitrin given, as we had previously obtained with adrenalin. On some animals there was an indication of a direct relation; but usually there were rather wide variations. These variations are probably due to the pituitrin not being so uniformly standardized as adrenalin. In fact, the manufacturing company states that the standardization of pituitrin requires more expert handling and greater skill than that of adrenalin.

Figure 1 shows the usual result of an injection of pituitrin. There is an immediate increase in the depth of respiration. This increase is followed by a shallowness and decrease in the rate of the respiratory movements.

In Figure 2 the effect of pituitrin on the circulatory system and respiration can be compared. It will be seen that the effects on the two systems occur synchronously; but the influence on respiration passes off before it does on the circulation.

Figure 3 shows another type of curve obtained. There is an increase in the depth of respiration which is followed by a shallowness and augmentation in the rate. Note the initial fall in blood pressure. In this cat all curves of blood pressure after the first injection, which was a rise, are characterized by this initial fall. This initial fall was also obtained in a few other experiments on cats.

The question arose as to whether the increase in the depth of respiration might be due to adrenalin being thrown out into the circulation as a result of introducing pituitrin. To throw light on this point, the adrenal glands were tied off in some experiments. Again pituitrin was introduced and the characteristic increase in the depth of respiration occurred.

In most cases, as shown in Figures 1 and 2, there was an increase in the depth of respiration lasting about one-third of a

FIG. 1. CAT

The introduction of 0.6 cc. of 1:25 pituitrin. The upper curve shows the effect on the movements of the diaphragm. Time, half minutes.

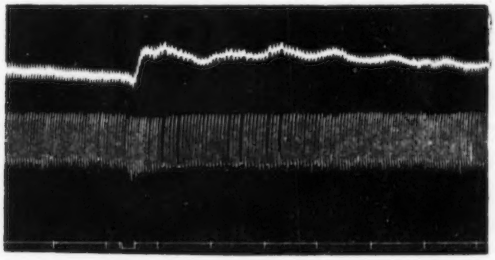

FIG. 2. CAT

The introduction of 0.9 cc. of 1:25 pituitrin. The upper curve shows the effect on blood-pressure and the middle curve the movements of the diaphragm. Time, half minutes.

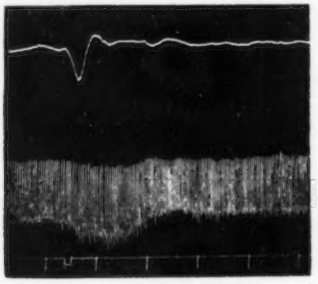

FIG. 3. CAT

The introduction of 1:2 cc. of 1:10 pituitrin. The upper curve shows the effect on blood-pressure and the middle curve the movements of the diaphragm. Time, half minutes.

minute. This was followed by shallowness and a decrease in the rate which usually lasted from two to five minutes.

In a few cases, as shown in Figure 3, the usual increase in the depth was followed by a shallowness and an augmentation in the rate of respiration. On the other hand in some of the experiments particularly on dogs the chacteristic response was a shallowness in the depth and an increase in the rate of respiration.

In our experiments the respiratory mechanism became fatigued or immune to pituitrin after a few injections had been given so that no response was elicited. A similar immunity has been noted by Howell and others in regard to the circulatory system.[6]

SUMMARY

1. The characteristic effect of pituitary extract on respiration is an increase in the depth followed by a shallowness and a decrease in the rate.

2. In some cases, however, the increase in the depth of respiration is followed by shallowness and an increase in the rate.

3. The effect of pituitrin on the respiratory mechanism occurs synchronously with that on the circulatory system. The effect, however, on respiration passes off sooner than that on circulation.

4. After a few injections of pituitrin, the respiratory mechanism becomes immune and the characteristic responses are not elicited.

[6] Howell: Journal Exper. Medicine; 1898, 3, p. 245. Also Vincent: Internal secretion and the ductless glands; London, 1912.

THE INFLUENCE OF TEMPERATURE ON THE RATE OF THE HEART BEAT IN AMBLYSTOMA EMBRYOS

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There can be no doubt that many physiological activities are affected by temperature in the same way that chemical reactions are, and that Van't Hoff's generalization in regard to the velocity of chemical reactions in relation to temperature applies to them. Numerous investigators have shown this to be the case. Snyder[1] has brought together the results of the work that had previously been done on the effects of temperature on the velocity of various physiological actions, and has calculated and compared the coefficients. In a recent paper[2] he has also shown that the rate of beat of the surviving mammalian heart is a logarithmic function of the temperature, and the coefficient for differences of 10°C., lies as a rule between 2 and 3. Rogers[3] found that Van't Hoff's law holds for the rate of beat of the intact heart of various vertebrates and invertebrates and Woodruff and Baitsell[4] proved that it holds for the rate of reproduction in Paramecium.

[1] Snyder, C. D.: A comparative study of the temperature coefficients of the velocities of various physiological actions. Amer. Jour. of Physiol., vol. 22, pp. 309–334, 1908.

[2] Snyder, C. D.: Is the rate of the surviving mammalian heart a linear or an exponential function of the temperature? Zeitschf. f. allg. Physiol., Bd. 15, S. 72–83, 1913.

[3] Rogers, C. G.: Studies upon the temperature coefficient of the rate of heart beat in certain living animals. Amer. Jour. of Physiol., vol. 28, pp. 81–93, 1911.

[4] Woodruff, L. L. and Baitsell, G. A.: The temperature coefficient of the rate of reproduction of Paramaecium aurelia. Amer. Jour. of Physiol., vol. 29, pp. 147–155, 1911.

Polimanti[5] subjected the embryos of a fish—Gobius paganellus—to different temperatures and timed the rate of the heart beat. He concluded that the rate approximately follows the law of Van't Hoff. The values of the coefficients for differences of 10°C which Polimanti obtained are very low, viz., 1.37 for the temperatures 16.5° to 25°−30°, and 1.36 for the temperatures 25°−30° to 35°−40°, giving an average of 1.365. These values are, however, in close agreement with those which Polimanti[6] obtained for the tortoise heart, viz., 1.20 for the temperatures 15° to 25°; 1.55 for the temperatures 25° to 35°; and 1.35 for the temperatures 35° to 45°, with an average value of 1.366 for the temperatures between 15° and 45°. Polimanti offers as an explanation of these small values the fact that in all of his work the heart was either left intact—in the fish embryos, or in situ—in the tortoise. When the average values of Q_{10} which have been given by Rogers for Fundulus embryos, viz., 3.081 for the temperatures 3.3° to 22.4° and of 1.521 for the temperatures 22.3° to 33.7°, and for toad-fish embryos, viz., 2.221 for the temperatures 9.9° to 20.7° and of 1.883 for the temperatures 20.7° to 30.6° are considered, Polimanti's results seem to demand some other explanation, and particularly so since Rogers' experiments were also carried out on the intact heart. In addition, Snyder's results on the terrapin heart are not in agreement with those of Polimanti on the tortoise, Snyder[7] finding an average coefficient of 2.89 for a range of temperatures between 0° and 40°, the values for some of the temperatures differing by 10° being as follows: 10.2 for the temperatures 0° to 10°; 3.5 for the temperatures 5° to 15°; 2.2 for the temperatures 10° to 20°; 2.1 for the temperatures 15° to 25°; 1.9 for the temperatures 20° to 30°;

[5] Polimanti, O.: Influences des agents physiques concentration, température sur l'activité du coeur embryonnaire des poissons. Jour. de Physiol. et de Pathol. gén., T. 13, pp. 797–808, 1911.

[6] Polimanti, O.: Sur quelques phénomènes observés en soumettant plusieurs parties du coeur à différentes températures. Jour. de Physiol. et de Pathol. gén., T. 9, pp. 768–783, 1907.

[7] Snyder, C. D.: On the influence of temperature upon cardiac contraction and its relation to influence of temperature upon chemical reaction velocity. Univ. California Publications, Physiol., vol. 2, pp. 125–146, 1905.

1.4 for the temperatures 25° to 35°; and 1.0 for the temperatures 30° to 40°. There are other values for the average coefficient of temperature for the tortoise heart, viz. Snyder[8] quotes a value of 2.5, for a range of temperatures between 2° and 32°, and Stewart[9] gives 2.6, for a range of temperatures between 6° and 37°.

Embryos of Amblystoma punctatum were being used for daily observations in connection with other work when it occurred to me that they offered excellent material for the study of the effects of different temperatures on the rate of beat of the intact heart. In the very youngest of these embryos in which there is any visual evidence that the heart is beating, the heart itself cannot be seen owing to the opaqueness of the epidermis, but the skin just ventral to the anterior limb bud pulsates with every heart beat, and thus gives a definite indication that the heart is beating and also of its rate. In older embryos the skin becomes transparent and the beating heart itself can be seen.

A series of experiments was therefore carried out to determine (1) whether evidence could be obtained that the rate of the heart beat of these embryos followed the law for the velocity of chemical reactions at different temperatures; (2) whether the results were different in embryos in which there was no probability that the heart had become connected with the central nervous system from those in which this connection had already taken place; and (3) if the values of the temperature coefficients obtained would not be more in accord with the results of others than with those of Polimanti.

The methods used for subjecting the embryos to the various temperatures and for the obtaining and keeping constant of these temperatures were very simple. The embryos to be experimented with, after they had been removed from the jelly, were placed in a small beaker containing 50 cc. of water or of 2: 10,000 chloretone solution. This beaker was floated in a battery jar

[8] Snyder, C. D.: 1908. l. c.

[9] G. N. Stewart.: The influence of temperature and of endocardiac pressure on the heart, and particularly on the action of the vagus and cardiac sympathetic nerves. Jour. of Physiol., vol. 13, pp. 59–164.

of 10 cm. diameter and 10 cm. deep which contained about 600 cc. of water. This jar was in turn placed in a stender dish of 22 cm. diameter and 8 cm. deep, in which about two liters of water were placed. The beaker was transferred from one such combination of battery jar and stender dish to another, the temperature in each being kept at the desired point. No thermostats were used, since it was found possible to keep the variations from the desired temperature within less than a degree Celsius, by means of a micro-burner and by placing crushed ice in the outer dish.

The embryos were subjected to temperatures from 5°C to 30°C and always continuously in a series differing by 5°. For each experiment three embryos were selected of approximately the same length and stage of development. Some time before they were subjected to the various temperatures the rate of the heart beat and the temperature of the water in which they were kept were noted, and again just before the experiment began. The embryos were then placed in the small beaker and this floated in water of 5°C. After 15 minutes the embryos, one after another, were transferred by means of a pipette to a watch glass placed on a "warm stage" attached to the stage of a Zeiss binocular microscope, which was kept as nearly as possible at the same temperature as the fluid from which the embryos had been taken. The rate of the heart beat was then timed by means of a stop watch for each embryo, after which they were transferred back to the beaker and this then floated in water of 10°C and so on up to 30°C. In order to show that the influence of the temperature was a reversible one after reaching 30°C the embryos were transferred in the reverse order back to 5°C. By this method it was hoped also to show that the results were free from gross errors, and since the results of the two series, ascending and descending, always agreed very closely, this is believed to be the case.

In the very earliest stages observed 10 beats were timed. It was, however, soon seen that with the development of the embryos the rate of the heart increased so much that it was impossible at the higher temperatures to time 10 beats with

any degree of accuracy, and accordingly 20 beats were thereafter timed. In the tables the number of beats timed is indicated.

Before proceeding to a description of the results of these experiments it will be best to give a brief account of the normal rate of the heart beat at the various stages of development.

The youngest embryos in which it can be seen that the heart is beating are between 6.5 mm. and 7.2 mm. long. The average rate of the heart beat at ordinary room temperatures at this stage is about 23 per minute. The rate of course varies in different individuals, the variations being due to the following factors (1) slight differences in the temperature of the water in which the different embryos are kept; (2) the activities of the embryos which tend to accelerate the rate; and (3) and most important, inherent differences in the various individuals themselves.

In a little later stage, in embryos measuring 7.5 mm. to 7.9 mm. the rate has increased to about 35 per minute. In embryos measuring 8.0 mm. to 8.8 mm. to about 40 per minute and in embryos 8.5 mm. to 9.3 mm. to 50 per minute. The rate thus continues to increase gradually, and in embryos that have just hatched which measure from 12.5 mm. to 13.8 mm. it is about 77 per minute. Soon after this, in embryos between 13.7 mm. and 15.8 mm. long, the mouth breaks through. There is at this stage still considerable yolk and the embryos probably do not begin to feed until this has been absorbed. At this stage the rate of the heart reaches its maximum, viz., 95 per minute. This high rate is, however, not very long retained and soon drops suddenly, for in embryos only a little farther advanced, viz., 16.7 mm. to 18.0 mm. long, which are feeding, the rate is only about 75 per minute. Hooker[10] has noted that in frog embryos which have been deprived of their nervous systems the maximum rate of the heart is higher than that of normal embryos. One of the explanations that he offers for this higher maximum in the operated embryos is that it comes at about the time when in the normal embryos the vagi become connected

[10] Hooker, D.: The development and function of voluntary and cardiac muscle in embryos without nerves. Jour. Exp. Zool., vol. 11, pp. 159–186, 1911.

with the heart. It is a very attractive idea to attempt to apply the same explanation for the sudden diminution seen here in the rate of the heart of normal embryos. The vagi probably become connected with the heart at about the time that the embryos begin to feed, and it may be assumed that coincident with the connection, or very soon after it is effected, there comes about the diminution in the rate of the heart beat.

The rate of the heart decreases but little again during the remainder of larval life. In embryos that are between 25 mm. and 30 mm. long the average rate per minute is 75 or a little less, and in larvae between 40 mm. and 50 mm. long it is between 65 and 73 per minute, with great variations caused by the activities of the individuals.

We see then that the heart of Amblystoma begins to beat at the rate of about 23 per minute, that it gradually increases to about 95 per minute and suddenly drops soon after the maximum is reached to about 75 per minute, this rate being held approximately through the entire later larval life.

The results of the temperature experiments may now be considered. Representatives of various stages of development were experimented with. The experiments on the younger embryos (up to 9.0 mm. long) were carried on in tap water. The animals did not move very much except when they were being transferred to and from the watch glass and when they were first placed in water of a higher or lower temperature. In order to do away with all movement and the chance of its having an accelerating effect on the heart the experiments on the later stages were carried out on embryos that had been narcotized in 0.02% chloretone. This drug renders the embryos absolutely immotile but does not very materially affect the rate of the heart beat during the time required for carrying out the experiment. Indeed, embryos that have been left for several days in chloretone show but a slight slowing of the rate of the heart beat.

The results of the experiments on the various embryos were all very uniform. Tables showing the results with the different stages have been given in order to demonstrate this. As one can see, in all cases, the temperature affected the rate of the heart

TABLE I

The influence of temperature on the rapidity of the heart beat in three Amblystoma embryos 8.0, 8.2, and 8.4 mm. long respectively. Time necessary for nine beats.

TEMPERATURE IN DEGREES CENTIGRADE	I	II	III	AVERAGE
5	64.8	63.6	70 2	66.2
10	39.8	37.4	38.6	38.6
15	19.8	23.2	21.9	22.6
20	13.8	15.2	13.6	14.2
25	10.0	11.0	10.2	10.4
30	7.8	8.2	9.2	8.4
25	8.0	10.6	10.8	9.8
20	13.0	14.0	13.8	13.6
15	19.6	23.0	22.8	21.8
10	37.0	36.6	35.0	36.2
5	62.6	63.6	64.0	63.4

Values of Q_{10}: $5°-15°=3.07$, $10°-20°=2.72$, $15°-25°=2.08$, $20°-30°=1.69$. Average value of $Q_{10}=2.39$.

TABLE II

The influence of temperature on the rapidity of the heart beat in three Amblystoma embryos 9.1, 9.3, and 8.9 mm. long respectively. Time necessary for nine beats.

TEMPERATURE IN DEGREES CENTIGRADE	I	II	III	AVERAGE
5	49.8	53.6	53.2	52.2
10	31.6	30.0	30.5	30.7
15	17.4	16.6	17.3	17.1
20	10.4	12.4	11.2	11.3
25	7.8	8.8	8.0	8 2
30	6.6	7.0	6.2	6 6
25	9.2	8.4	6.4	8 0
20	11.6	10.2	10.6	10.8
15	18.4	17.6	19.8	18.6
10	29.4	28.6	27.8	28.6
5	49.6	52.0	47.8	49.8

Values of Q_{10}: $5°-15°=3.05$, $10°-20°=2.72$, $15°-25°=2.09$, $20°-30°=1.71$. Average value of $Q_{10}=2.39$.

TABLE III

The influence of temperature on the rapidity of the heart beat in three Amblystoma embryos 13.5, 13.1, and 12.5 mm. long respectively. Time necessary for nineteen beats.

TEMPERATURE IN DEGREES CENTIGRADE	I	II	III	AVERAGE
5	74.6	72.7	78.5	75.3
10	43.4	42.2	40.1	41.9
15	25.8	25.2	23.2	24.7
20	15.1	15.8	15.4	15.4
25	10.3	11.6	10.7	10.9
30	9.5	8.8	9.2	9.2
25	10.0	10.9	10.4	10.4
20	15.8	15.7	16.6	16.0
15	25.9	25.4	24.0	25.1
10	47.8	46.3	47.9	47.3
5	75.8	79.7	78.6	78.0

Values of Q_{10}: 5°–15°=3.05, 10°–20°=2.72, 15–25°=2.27, 20°–30°=1.67. Average value of Q_{10}=2.43.

TABLE IV

The influence of temperature on the rapidity of the heart beat in three Amblystoma embryos 25.6, 27.0 and 24.0 mm. long respectively. Time necessary for nineteen beats.

TEMPERATURE IN DEGREES CENTIGRADE	I	II	III	AVERAGE
5	74.5	77.8	78.9	77.1
10	45.2	46.0	48.2	46.5
15	23.8	24.7	24.5	24.3
20	16.5	16.0	17.6	16.7
25	10.9	10.7	11.8	11.1
30	8.2	8.8	9.5	8.8
25	10.0	10.8	11.4	10.7
20	16.3	14.7	15.8	15.6
15	24.0	24.8	24.8	24.5
10	42.8	41.9	41.4	42.0
5	73.7	74.5	70.4	72.6

Values of Q_{10}: 5°–15°=3.17, 10°–20°=2.78, 15°–25°=2.19, 20°–30°=1.90. Average value of Q_{10}=2.51.

beat, and its frequency is undoubtedly a function of the temperature, and a logarithmic and not a linear one. Curves have been constructed for all of the experiments, but since they are perfectly regular and show nothing novel, they are omitted. The same results were obtained with the hearts of embryos in which there were no nervous parts as with those in which the nerves had undoubtedly become connected with the heart. Without going any farther into this matter it may be stated briefly that the evidence certainly points to the view that it is the heart muscle itself which is affected by the changes in temperature and not the regulatory nervous portions, which, it might be assumed, brought about secondarily the variations in the rate of the heart.

Beneath the tables have been placed the values of the temperature coefficients for differences of 10°C. These values were calculated by using the formula given by Snyder.[11] This formula has the form $Q_{10} = \left(\frac{K_1}{K_0} \right)^{\frac{10}{T_1 - T_0}}$ in which Q_{10} is the coefficient of the increase in reaction velocity for a rise of 10°C., and the symbols K_1 and K_0 represent the rates of beat of the heart at the temperatures T_1 and T_0 respectively. It need hardly be mentioned that for differences of 10° this formula is reduced to nothing more than the rate at the higher temperature divided by that at the lower.

These values of Q_{10} show one of the general characteristics of the temperature coefficients of physiological processes, viz. that they are greater for the lower and smaller for the higher ranges of temperature (Snyder).[12] With particular reference to the rate of the heart beat, as Rogers[13] has already pointed out, there is a clear indication that the acceleration of the heart beat decreases steadily as we pass from the lower to the higher temperatures.

[11] Snyder, C. D.: 1908. l. c.

[12] Snyder, C. D.: On the meaning of variation of magnitude of temperature coefficients of physiological processes. Amer. Jour. of Physiol., vol. 28, pp. 167–175, 1911.

[13] Rogers, C. G.: 1911. l. c.

The values of Q_{10} for the different ranges of temperature for all of my experiments have been averaged and are as follows: 3.06 for the temperatures 5° to 15°; 2.58 for the temperatures 10° to 20°; 2.14 for the temperatures 15° to 25°; and 1.75 for the temperatures 20° to 30°. The general average of all these values is 2.38.

In addition to the experiments already described others were carried out to test the effects of temperature on the rate of the heart beat of embryos that had been kept for considerable lengths of time at low or high temperatures. The results were on the whole unsatisfactory. It was soon found that, when embryos of various stages of development are kept at a temperature between 0°C. and 4°C. in a constant temperature room for 24 hours or more, serious abnormalities in form and function result. The pericardium and body cavity become swollen and distended, the skin quite thin and transparent, and the heart beats arhythmic and periodic. Immediately after these embryos are removed from the constant temperature room the contraction of the heart is so feeble that it can hardly be observed to be beating at all. In a few minutes, allowing the temperature of the water in which the embryos are kept to gradually rise, the heart beat becomes stronger. The average rate of beat of these hearts, after the temperature of the water has risen to that of the room, is always lower than that of the usual average, and this effect lasts for several hours. When such embryos are placed, immediately after being taken from the cold room, in water of various temperatures as in the other experiments, the rate is always lower for each temperature than is that of the normal, although the values of the temperature coefficients are about the same.

Subjecting embryos to a temperature of 30°C. for 24 hours or more also produces abnormalities, and of greater magnitude and duration than those produced by subjection to low temperatures. In addition to the body cavity and pericardium becoming swollen and distended these larvae always show a marked curvature, the head and tail being bent sharply upwards. Such embryos also show the abnormalities in the heart beat. The rate of

the heart beat is of course very much increased in these embryos, but when the water in which they are kept gradually cools down to room temperature the rate does not show a very marked difference from that of embryos not previously exposed to prolonged high temperature. When these embryos are transferred immediately from water of 30°C. to water of 5°C. and run through the series up to 30°C. and back again to 5°C. the average rate of the heart beat for the various temperatures does not differ very much from that of embryos which have been kept at ordinary room temperatures.

Very often in these embryos the rate is much retarded owing to the abnormalities produced by the high temperature. Quite a number of them die after two or three days. A shorter exposure to the high temperature results in a reduced mortality, but also in a much reduced deviation in the results from those obtained with animals not so exposed.

Taken all in all the experiments dealing with prolonged exposure to low and high temperatures do not show a very marked effect. Loeb and Ewald[14] obtained much more definite results and showed a marked effect on the values for the different temperatures and one which could be noted for several hours afterwards.

My experiments, but for those on the very oldest embryos, were completed when this paper by Loeb and Ewald came to my notice. These investigators show that the rate of the intact heart of Fundulus embryos is clearly a logarithmic function of the temperature. I have calculated the temperature coefficients and constructed curves from their data which show a close similarity to those of my own for the embryos of Amblystoma. Since my experiments were carried out on another animal, and since they were carried out on young and old embryos this close agreement in the general results renders them of additional interest.

In addition to the experiments with temperature quite a number were carried out subjecting embryos to various solu-

[14] Loeb, J. und Ewald, W. F.: Die Frequenz der Herztätigkeit als eindeutige Funktion der Temperatur. Biochem. Zeitschr. Bd. 58 S. 177–185. 1914.

tions, in order to see if any effect on the rate of the heart beat could be noted. Among the solutions used were the following: a Ringer, 0.6 per cent NaCl, 0.3 per cent NaCl, 0.2 n NaCl, 0.1 n NaCl, 0.8 per cent KCl, 0.4 per cent KCl, 0.2 n KCl, 0.1 n KCl and 0.02 per cent chloretone. In all, except the last, development will proceed, in most cases abnormally, up to a certain stage, after which growth ceases, though the embryos may live for a considerable time after this, and even exhibit degenerative processes before they finally die. In all of the solutions, excepting again chloretone, the rate of the heart beat as well as its strength is impaired, long before the death of the embryos comes about. These experiments are not yet complete, and it is hoped to continue them next spring.

SUMMARY

1. The rate of the heart beat of Amblystoma embryos is a logarithmic function of the temperature.

2. The acceleration of the heart beat is greater for lower temperatures than for higher.

3. The value of the coefficients of temperatures for differences of 10°C. (Q_{10}), are as follows: 3.06 for 5°C. to 15°C.; 2.58 for 10°C. to 20°C.; 2.14 for 15°C. to 25°C.; and 1.75 for 20°C. to 30°C. The average value of Q_{10} for the temperatures 5°C. to 30°C. is 2.38.

4. Van't Hoff's law for the velocity of chemical reactions in relation to the temperature holds for the rate of the heart beat of these embryos.

BODY TEMPERATURE AND PULSE RATE IN MAN AFTER MUSCULAR EXERCISE

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The cardio-acceleration attending muscular exercise presents two distinct phases. The first of these is an immediate and pronounced increase in rate which Hering,[1] Hunt,[2] Bowen,[3] Johansson[4] and others[5] have shown to be mediated through the nervous system and to operate by depression of the cardio-inhibitory center.

The second is a persistent augmentation which endures a variable but considerable time after cessation of the exercise and for which there is as yet no wholly satisfactory explanation. This persistent acceleration has been studied by Johansson[6] and by Mansfeld.[7] Their results are in many respects conflicting and their conclusions are largely non-concordant. Both observers take the view that the quickened rate is maintained by the blood flowing through the heart itself. Johansson would have metabolic products of muscular activity the immediate agents of acceleration, while Mansfeld considers the increase in the temperature of the blood the efficient source of the quickened action. A third possibility is considered and rejected by Gasser and Meek,[8] namely that muscular exercise may be accompanied by an outpouring of adrenalin which, in turn, accelerates the heart.

[1] Hering: Archiv für die gesammte Physiologie, 1895, lx, p. 483.
[2] Hunt: This journal, 1899, ii, p. 464.
[3] Bowen: Contributions to Medical Research, Ann Arbor, 1903, p. 462.
[4] Johansson: Skandinavische Archiv für Physiologie, 1895, v, p. 20.
[5] Gasser and Meek: This journal, 1914, xxxiv, p. 48.
[6] Johansson: Loc. cit.
[7] Mansfeld: Archiv für die gesammte Physiologie, 1910, cxxiv, p. 598.
[8] Gasser and Meek: Loc. cit., p. 69.

There are two distinct factors in the general problem. The first of these is whether the stimulus for the persistent acceleration acts by way of the central nervous system or upon the heart directly; the second, whether metabolites, blood temperature, or some other condition serves as the actual stimulus. Although with reference to the first of these factors Johansson and Mansfeld were agreed in considering the action to be directly upon the heart, their evidence may well be reëxamined in view of the importance of the problem.

Johansson's method of showing that the effect was a direct cardiac one was to observe the acceleration that followed activity of the hind limbs from tetanization of the lumbar cord before and after cutting the vagi and accelerators.[9] He experimented upon dogs. Five experiments in his entire paper may be used as a basis for conclusion, although the acceleration he obtained in these experiments subsided so quickly that there is some reason to doubt whether it truly represented the persistent type. In tables V *a* and XVIII of Johansson's paper, the results of exercise in dogs with intact cardiac nerves are reported. In tables IX *a* and the latter part of V *a* dogs with vagi cut and accelerators apparently intact were used. Tables XVI, XVII, and the latter part of XVIII give the results when all cardiac nerves were severed. To make our comparisons from these tables fair, the most rapid rates during rest periods were compared with the most rapid during exercise. In two experiments with all cardiac nerves intact, in one of which (Table XVIII) only *weak tetanization* was used, the average actual acceleration was 9 beats per minute, the average percentage acceleration 11.5. In four experiments with the vagi cut and the accelerators intact the average actual acceleration was 8 beats per minute, the average percentage acceleration 3.5. In eight tests in which all cardiac nerves were cut the average actual acceleration was 9.2 beats per minute, the average percentage acceleration 6.4.

As opposed to these results of Johansson, Mansfeld,[10] working chiefly upon dogs, with a few cats, reports striking accelerations

[9] Johansson: Loc cit., pp. 59–61.

[10] Mansfeld: Loc. cit., p. 603.

when the cardiac nerves were intact, and relatively little acceleration when one or both were cut. His figures are: for twelve experiments with nerves intact an average actual acceleration of 40 beats per minute, giving an average percentage acceleration of 23; for 15 experiments with one or both nerves cut an average actual acceleration of 6.3 beats per minute, amounting to an average percentage acceleration of 3.8.

Mansfeld studied, further,[11] the effect upon the heart rate of various metabolic products, including carbon dioxide and extracts of fatigued muscles, and found that these had no accelerating influence. These results have been confirmed by Peterson and Gasser[12] upon isolated cats' hearts.

The evidence thus far cited would seem to point to the central nervous system rather than to the heart as the region of stimulation for the persistent cardio-acceleration of exercise. Why, then, does Mansfeld, in the face of his own results, refer the point of stimulation to the heart? His reason is given in two experiments reported on page 609 of his paper, already cited, in which he obtained in dogs with both carotid arteries and both vertebral arteries tied off striking accelerations (70 per cent and 55 per cent) upon tetanizing the lumbar cord. These experiments would be more convincing had Mansfeld selected other animals than dogs for his experiments, for Leonard Hill[13] has shown that in dogs an adequate brain circulation usually persists even after both carotids and both vertebrals have been occluded. Since Mansfeld believed that these experiments afforded a valid demonstration of acceleration brought about in the absence of brain circulation, he was led to interpret his former results on the basis of a reflex mechanism in which the receptors affected by the blood were in the heart itself. His negative results with circulating metabolites together with an observed variation in pulse rate with changes in the temperature of the blood flowing through the heart convinced him that the stimulating agent in his reflex mechanism was the temperature of the blood.

[11] Mansfeld: Loc. cit., p. 610.

[12] Peterson and Gasser: This journal, 1914, xxxiii, p. 301.

[13] Leonard Hill: The cerebral circulation, London, 1896, p. 125.

When we recall the demonstrated susceptibility of certain bulbar centers, notably the respiratory and sweat centers, to influences brought to them by way of the blood, Mansfeld's theory, which denies to the cardiac centers similar susceptibility, and substitutes therefor a cumbersome reflex mechanism, would seem to require for its acceptance much more certain proof than either he or Johansson has afforded. Indeed in view of Hill's observation cited above, we are inclined to explain Mansfeld's results by assuming them to be due to an influence of the blood on the bulbar cardiac centers.

Inasmuch as a great part of the work which has seemed to exclude metabolites as sources of cardiac stimulation has been directed at the isolated heart, we feel that in returning to the bulbar centers as possible seats of stimulation the question of the nature of the stimulus is reopened.

The directly conflicting experimental results of Johansson and Mansfeld seem to us to show that their method of approaching the problem is unlikely to yield in the hands of other investigators data more conclusive than theirs. We have sought, therefore, for some other means by which pertinent evidence might be gained. A further reason for abandoning their method is that in experimental animals under their conditions the cardio-accelerations observed are of such short duration as compared with those occurring in human subjects as to give room for doubt as to whether they represent precisely similar phenomena.

Experiments. The experiments which we have to report were performed upon men. Cook and Pembrey[14] have shown that the persistence of cardio-acceleration may vary greatly with the condition of "training" of the subjects. In accordance with this observation, which our experience confirms completely, we made observations upon an athlete, L., in good condition as regarded training, and upon an untrained man, M., in good general health. If Mansfeld's theory is correct, that the persistent acceleration of the heart after exercise depends on the temperature of the blood, careful comparisons of body temperature and pulse rate

[14] Cook and Pembrey: Journal of Physiology, 1912, xlv, Proceedings, p. 1.

after exercise should show an inter-dependence between them. Moreover, the striking prolongation of the acceleration sometimes seen in untrained as compared with trained men should be correlated with a definite difference in the period of heightened body temperature.

The form of exercise we adopted was running up and down stairs, a form well calculated to bring about in a few minutes of exercise a markedly persistent cardio-acceleration. Our method of determining pulse rates was by direct counting at the wrist for twenty seconds at one, two, or five minute intervals according to the stage of the experiment. Our subjects had undergone previous pulse countings with sufficient frequency to be free from the disturbing psychical influence that sometimes attends pulse counting. All readings were taken with the subject lying comfortably supine on a couch.

The problem of accurate body temperature determination presented some difficulty. On the basis of the work of Benedict and Slack[15] we decided on the well-closed axilla as the region to be used.

For registering axillary temperatures we employed a standard mercury thermometer graduated in tenths of a degree centigrade, and with a scale so long that with the aid of a lens approximate readings to hundredths of a degree could be made. The stem of this thermometer was laid along the naked body with the bulb in the axilla. In some, but not in all, of our experiments surgeon's plaster strips were used to hold the thermometer in place. Pledgelets of absorbent cotton were placed on either side of the thermometer bulb; the arm was brought closely against the side of the body, and confined there by a large towel bandage about the shoulder. The long stem of the thermometer projected beyond the bandage along the front surface of the forearm. Previous tests of the thermometer had shown that it reacted to changes of temperature very promptly, quite promptly enough to enable it to follow accurately changes in axillary temperature after

[15] Benedict and Slack: A comparative study of temperature fluctuations in different parts of the human body, Carnegie Institution of Washington, Publication number 155, Washington, 1911.

equilibrium had once been established. Since Benedict and Slack[16] had shown that axillary temperature reaches equilibrium slowly, we never began an experiment until three or four temperature readings, taken at two-minute intervals, agreed within 0.01°C. During this preliminary period the subject lay upon a couch while pulse and temperature readings were made. When the observer was satisfied that both pulse and temperature had held steady for at least five minutes the subject was sent upon his run. In the case of the untrained man three minutes of exercise were taken at top speed; about half the time was spent in running along hall-ways, and half in running up or down stairs. The trained man ran for about twice as many minutes. He, again, divided the time roughly into equal intervals of running in hall-ways and on stairs. With the completion of the exercise the subject returned to his couch, on which he lay supine, and pulse and temperature readings were resumed as quickly as possible.

In both our subjects, and also in two other subjects, G. and La, on whom pulse readings but not temperature readings were made, the pulse rate fell within three or four minutes after cessation of the exercise to approximately 100 per minute, and from that time the slowing became markedly more gradual. We are inclined to consider this first rapid decline in rate as the result of the withdrawal of those influences to which are due the immediate acceleration;[17] and the rate of beat which prevails with slow decline thereafter to be due to that persistent influence whose nature is the subject of this inquiry.

The experiment first to be reported was performed on April 28, 1913 upon L. as subject. The day happened to be warm and exceptionally humid. This latter fact was strikingly evidenced by the streams of sweat that coursed over the subject's body after the exercise. The preliminary readings were: pulse 57 per minute; axillary temperature 36.73°C. Four minutes after completion of the exercise the pulse had dropped from a maximum of 160 per minute to 99 per minute, while the temperature stood

[16] Benedict and Slack: Loc cit., p. 43.

[17] See Martin and Gruber: This journal, 1913, xxxii, p. 315.

at a maximum reading of 37.42°. During the next twenty minutes both pulse rate and body temperature declined steadily, and in such parallel fashion as to suggest an inter-relation between them. At the end of this time the pulse rate was 70 per minute and the temperature 37.15°C. After this, during the next forty minutes the pulse rate held steady with minor fluctuations around 70 per minute, while the temperature continued to drop. At the end of the period it stood at 36.95°. The experiment was then discontinued, although neither pulse rate nor temperature had returned to its original value.

This experiment, although not by itself conclusive in any direction, contains several suggestive features. It differed from our ordinary experiences with subject L. in that the pulse rate continued high for nearly an hour after the exercise. There was a severe handicap upon the heat-loss mechanism in the exceptional humidity of the day, which interfered strikingly with sweat-evaporation, and which showed itself in a much slower rate of cooling of the body than in any of our other experiments. The body temperature an hour after the exercise was 0.2°C. higher than at the beginning of the experiment.

These observations suggested to us the desirability of accentuating the comparison between pulse rate and body temperature by bringing about experimentally modifications in the rate and amount of change of the latter so as to see whether the former would show corresponding variations. The experiments which seem to us conclusive were carried out in accordance with this plan.

A method of prolonging the period of heightened body temperature following exercise which proved successful was the administration of a cold-bath. Currie[18] showed that there is a slight rise in buccal temperature immediately after a cold bath. The same was later shown by Liebermeister[19] to be true of axillary temperature.

[18] **Currie**: 1794, quoted from Pembrey: Schäfer's text-book of Physiology, Edinburgh, 1898, i, p. 818.

[19] **Liebermeister**: Archiv. für Anatomie, Physiologie, und wissenschaftliche Medizin. Leipzig, 1860, pp. 520, 589.

A representative experiment was as follows: the subject L., clad only in running breeches, with the thermometer secured in the axilla in the manner described above, underwent the usual preliminary observations, performed the stated exercise, and returned to the couch on which he lay while observations were made. At minute intervals for six minutes pulse and temperature readings were taken, the subject being covered meanwhile with a blanket. He was then quickly disrobed completely and stepped into an adjoining room, where for five minutes a stream of cold water was kept playing on his body, with care to avoid the region of the thermometer. He was then dried hurriedly and returned to his couch, where he lay under the blanket for three minutes while readings of pulse and temperature were taken. During the following three minutes another cold shower was administered, after which the subject lay quietly under his blanket for fifty minutes, pulse and temperature being observed at regular intervals throughout. The results of this experiment were as follows: the pulse before exercise was 63 per minute, the axillary temperature was 36 83°C. Six minutes after the exercise the pulse had dropped from a maximum rate of 146 per minute, to 102; the temperature was 37.45. Immediately after the first cold bath the pulse was at 90 per minute, and the temperature at 37.4°. During the three minutes before the second bath the pulse dropped to 82 per minute, while the temperature rose to 37.62°. Two minutes after the second bath the pulse stood at 73 per minute, while the thermometer registered the same as before this bath, 37.62°. During the next fifty minutes the pulse rate averaged about 70 per minute, with slight fluctuations on either side the average; during this same period, beginning four minutes after the conclusion of the second bath, the axillary temperature fell steadily to a minimum of 36.76°, reaching this minimum only after forty minutes. Curves showing the course of pulse rate and of axillary temperature in this experiment are given in figure 1. That the cold bath is a successful means of prolonging the period of heightened body temperature is evidenced from the fact that in this experiment the maximum temperature was recorded twenty-two minutes after cessation of exercise,

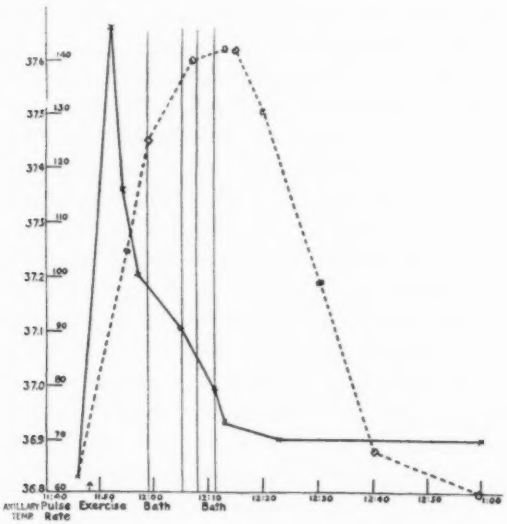

Fig. 1. Curves illustrating the course of axillary temperature and pulse rate after exercise and cold shower baths. The continuous line represents pulse rate; the dotted line, axillary temperature.

while in other experiments on the same subject, where the cold bath was not used, the temperature drop began in about six minutes after exercise.

In this, as in all our experiments with subject L., the pulse returned nearly to its original rate and became steady in about twenty minutes after the exercise. So far as we can see, the behavior of the heart was not modified in the slightest degree by a procedure which kept the temperature high for a considerable period.

While this experiment by itself seems to us to afford sufficient proof that heightened body temperature is not responsible for the persistent cardio-acceleration of exercise, we desired to reinforce it if possible by observations of the contrary situation, in which the lowering of body temperature is hastened artificially. A method of bringing this about which proved efficacious was to expose the subject with uncovered body, or only the thinnest of

covering, to a brisk current of air, such as is generated by an electric fan. In an experiment of this sort with subject L. the body temperature returned to its initial value twenty-two minutes after the cessation of exercise, as against thirty to fifty minutes in other experiments. The pulse returned to its original rate in twenty minutes, as it did usually with this subject, so that there was, thus far, apparent agreement between the two phenomena. Beyond this point there was, however, this difference, that while the pulse rate continued substantially constant for forty minutes, when the experiment was terminated, the temperature *continued to fall*, becoming finally steady, one hour after the exercise, at a point 0.4°C. below the original value.

The method of cooling the body by a direct current of air proved so efficacious that it seemed desirable to test its effect on an untrained subject, in whom the return of the heart to its normal rate would be slower. Subject M. was used for this experiment. Perhaps on account of the smaller amount of exercise taken, the rise in axillary temperature was relatively slight, from 37.30° to 37.45°C. The heart was markedly accelerated, going from an initial rate of 69 per minute to 144, and dropping back in four minutes to 100, whence it declined slowly to a rate, in forty minutes, of 81 per minute. The body temperature began to fall within two minutes after the completion of the exercise, and in ten minutes, while the pulse rate was at 96 per minute, had returned to its initial value. During the next forty minutes the temperature continued to fall, reaching finally a point 0.6°C. below that shown before the exercise began. In spite of this marked drop in temperature the pulse continued to behave as usually in this untrained subject, remaining persistently more rapid than before the exercise.

Discussion. If our results are trustworthy these experiments seem to show clearly that whatever may be the mechanism of the persistent cardio-acceleration of exercise, body temperature has little to do with it. The trustworthiness of our results depends, of course, on the validity of our experimental methods; we wish, therefore, to consider these briefly. There are possibilities of error both in pulse rate determinations and in body temperature

measurements. The method of pulse rate determination by counting at the wrist is accepted as valid for all ordinary purposes. Our problem called for information as to the general trend of pulse rate variations, rather than for accurate knowledge of minute momentary fluctuations. Wrist counting seemed to us, therefore, sufficiently accurate for our needs. The factor of psychic disturbance we eliminated so far as possible by accustoming the subjects to the procedure through numerous preliminary tests. There seems good reason to believe, moreover, that psychic influences may be less in control during the period of acceleration immediately following exercise than at other times.

The precise determination of body temperature we know from the studies of various observers[20] to be a matter of considerable difficulty. Benedict and Slack[21] have indicated the most satisfactory technique for such determinations and have also discussed the advantages and disadvantages of other methods. According to their work, the most serious objection to the axilla as the site of temperature measurements, is the difficulty of keeping it well closed, especially when bodily movements occur. In our experiments the arm on the side to which the thermometer was applied was secured as firmly as possible. The subject, moreover, lay quiet during the period when observations were being made. While exercise was actually in progress there was doubtless some dislocation of the thermometer bulb.

A standardized mercury thermometer, if allowance is made for its lag, is recognized as a highly accurate means of determining temperature. The sources of error pointed out by Benedict and Slack[22] for *clinical* thermometers do not apply to instruments of the ordinary type.

For the purpose of our experiments actual temperatures were unimportant if the degree of variation from time to time was accurately indicated. A good mercury thermometer carefully secured in the axilla, even though it might fail to give the blood temperature precisely, should vary from the actual temperature by

[20] See Benedict and Slack: Loc. cit.
[21] Benedict and Slack: Loc. cit., p. 72.
[22] Benedict and Slack: Loc. cit., p. 43.

a fixed ratio so long as its position remains unaltered. Moreover, such errors as enter from unavoidable shifts in thermometer position are not likely to amount to much more than 0.1°C., whereas our conclusions are based on temperature differences of 0.4°C. or more.

If our contention be sustained that the error of our method is too small to account for the wide divergence of pulse rate curves and body temperature curves which we have reported, the conclusion seems to us unavoidable that some other cause than the rise in blood temperature must be sought as the sustaining agent in the persistent cardio-acceleration which follows muscular activity.

Metabolic activity and pulse rate.—That variations in metabolic activity in man are accompanied by variations in heart rate has been emphasized in various contributions; notably the admirable series from the Nutrition Laboratory of the Carnegie Institution of Washington.[23, 24, 25] Whether heightened metabolic activity is the *cause* of an augmented pulse, or whether both depend on a common antecedent stimulus does not appear, although critical study of the experimental data, notably of Benedict and Cathcart (loc. cit. tables 61 and 64) suggests rather a common cause than strict dependence of one on the other. That this cause, whatever it may be, operates in the special case of the persistent acceleration after exercise appears probable from the observations of Benedict and Cathcart (loc. cit., p. 163, et seq.) who found that the metabolism, as measured by the oxygen absorption, continued high after exercise for periods comparable with those of augmented heart action. That these effects are dependent on an increase in the temperature of the blood seems to us, from our observations cited above, altogether unlikely.

[23] Benedict, F. G.: Influence of inanition on metabolism. Carnegie Institution of Washington, Publication number 77.

[24] Benedict and Carpenter: Metabolism and energy transformations of healthy man during rest. Idem, Publication number 126.

[25] Benedict and Cathcart: Muscular work. Idem, Publication number, 187.

SUMMARY

Careful comparisons of axillary temperature and pulse rate after muscular exercise in trained and untrained men fail to show the degree of parallelism to be expected if Mansfeld is correct in his view that the persistent cardio-acceleration following exercise is the result of heightened blood temperature.

The conclusion is drawn that some other explanation must be sought for the phenomenon.

THE EFFECT OF X-RAYS ON THE ACTION OF CERTAIN ENZYMES

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During the progress of certain investigations[1] on the effect of X-rays on the development of eggs of the snail *Planorbis*, it became desirable to know something of the special effect these rays have on enzymes. It is not possible, of course, in the present state of our knowledge, to obtain information as to the specific effect on the particular enzymes which are involved in the activities of the eggs; but in the event that consistent effects can be obtained on those enzymes which may be extracted in a state of relative purity, it may be inferred that the laws which govern their action would also apply to the action of others less easy to study.

Although the available literature on the biological action of X-rays has been carefully examined, the writer has found but one series of experiments which bears directly on the question as to the effect of these rays on enzymes. Richter and Gerhartz[2] studied the effects of the rays on rennin, yeast, pepsin, pancreatin, and papain, as well as on adrenalin. According to these authors, their experiments showed that the radiation by X-rays affect ferments in no way, and that the variations from this result lie within the bounds of experimental error. But the report of the experiments upon which their conclusions are based does show differences in the behavior of the radiated and non-radiated substances. Whether these differences, slight as they are in some cases, can be attributed to experimental error is, to the mind of

[1] Richards, A.: The effect of X-rays on the rate of cell division in the eggs of Planorbis. Biol. Bull., xxvii, 1914.

[2] Richter and Gerhartz: Ueber die Einwirkung der Roentgenstrahlen auf Fermente. Berl. Klin. Wochenschr., Bd. 45, 1908.

the present writer, very questionable. In some of the experiments no control was given, and these should not, therefore, be taken into consideration in drawing conclusions. It is worthy of note, that if these experiments, which show decisive evidence neither for nor against the conclusion stated, be neglected, all of the cases reported by Richter and Gerhartz, with the exception of only one, gave results consistent with those presented here and are open to the same interpretation as that given below, namely that weak radiation accelerates the action of the enzyme while strong radiation inhibits it. The authors measured the increase in blood pressure after the injection of radiated and of non-radiated adrenalin. In the first experiment the blood pressure at the time of injection of adrenalin radiated for twenty minutes was 80; it thereupon rose to 92, but only after two minutes; while in the control unradiated adrenalin caused a rise to 120 in one-half minute. That is, the radiated adrenalin was much less active than the non-radiated. In their second experiment the duration of the exposure was only five minutes and the increase in the blood pressure was from 60 before injection to 120 afterwards in the case of both radiated and control adrenalin. The authors regard this experiment as negative, but it is consistent with the interpretation given below, according to which the radiation of five minutes' duration was probably of the "non-effective" type, being too weak to inhibit, and yet strong enough to prevent the acceleration. No further experiments are reported in which the exposure of the adrenalin was of short duration. In the other experiments where adrenalin was used and data from a control are given, the exposed agent was less active as measured by blood pressure changes than the non-radiated adrenalin.

The same investigators experimented with a number of ferments. Rennin, radiated for five minutes, and also the control, coagulated milk in one and three-quarters hours. (Was this a "non-effective" radiation?) Again milk was less completely coagulated in twenty minutes by rennin exposed for ten minutes than by that which was unradiated. Five minutes radiation for yeast was non-effective. Dry pepsin radiated five minutes on

each of two successive days entirely failed to dissolve fibrin in three hours, while the non-radiated ferment partly dissolved it. In another experiment dry pepsin was given long radiation and its effect on the digestion of casein tested. After six hours digestion at 36°C. as determined by the Kjeldahl method, the mixture with radiated pepsin gave 0.088 per cent N. and the control 0.067 per cent N. After twenty-one hours the mixture with radiated pepsin gave 0.212 per cent N. and the control 0.220 per cent N., a result consistent with those described below. It is true of course that these differences are very slight. In a third experiment pepsin was radiated twenty minutes on each of three successive days. Its digestion of casein as determined by the Kjeldahl method produced 0.075 per cent N. as contrasted with 0.068 per cent N. produced by the non-radiated pepsin. This case is the exception already mentioned. All of these experiments are to be criticized since dry pepsin was used for radiation rather than a solution. The digestion of casein by radiated and non-radiated pancreatin gave similar results, the unradiated ferment being slightly more active than the radiated. Casein digestion by papain also showed the same sort of results. A perusal of these experiments and their outcomes, therefore, can leave little doubt that radiation does effect enzymes. It is a different matter whether there is justification for the authors' claim that these results are all within the range of experimental error, but the fact that these variations, slight as they are, occur consistently in one direction seems to refute that claim. These considerations, when brought into relation with the experiments here reported, the writer believes, give additional support for the conclusion stated below.

Radium rays which are in general comparable with the X-rays in their actions have been thought to be the cause of quite marked changes in the course of enzymotic action. It has been rather generally assumed the effects produced by these two agents on protoplasm is in no small measure due to some special effects on the enzymes in the tissue in question. Certain investigators also claim to have found evidence that enzymes are activated by radium radiations.

The recent biological studies of the effects of radiation on animal and plant cells have given rise to two quite distinct theories to account for the clearly marked and unquestioned effects which have been observed. The theory of O. Hertwig[3] and his coworkers, called by them a "biological hypothesis," is that the effect is definitely dependent on the chromatin, and evidence in support of this position is found in the behavior of eggs and sperm of frogs and sea-urchins when subjected to radiation. Their cytological studies as well as those of Packard[4] on *Nereis* and of several other workers show conclusively that profound changes in the chromatin undergoing division are wrought by radiation; and the writer also has observed confirmatory evidence in the *Planorbis* eggs which were exposed to the X-rays. Opposed to Hertwig's hypothesis is the older theory of Schwarz[5] and others that the injurious effects are due to the breaking down of lecithin. Lecithin is found quite generally in many kinds of cells, and its decomposition is easily conceived to be attended with marked results on the tissues in question. Schwarz found evidence for the view that lecithin is decomposed in the egg and that eggs rich in lecithin content are most effected by radiation.

Neither of these theories is found satisfactory by Packard to explain the observed facts and he suggests "that the radium radiations act indirectly on the chromatin and protoplasm by activating autolytic enzymes which bring about a degeneration of the complex proteids, and probably by affecting other protoplasmic substances in the same manner."

From the foregoing, it will be seen that the changes in the action of the enzyme caused by radiation bear directly upon the solution of the broader biological questions of the relation of the tissues and their functions to radiation. Many observations on the effects produced would be best explained on the assumption

[3] Hertwig, O., G., and P.: Several papers in Arch. f. Mikr. Anat., Bd. 77, Abt. 2, 1911, and Bd. 79, Abt. 2, 1912.

[4] Packard, Charles: The effect of radium radiation on the fertilization of Nereis. Jour. Exp. Zoöl., v. 16, 1914.

[5] Schwarz, G.: Ueber die Wirkung den Radiumstrahlen; eine physiologischechemische Studie am Hühnerei. Arch. f. Physiol., Bd. 100, 1903.

that radiation causes an acceleration of the enzyme, but clear-cut evidence in favor of the view is not abundant in the case of radium, and in the case of X-rays is entirely wanting.

It should be noted in this connection that the writer sees by no means a complete explanation of the effects of radiation on cells and tissues in the results of the experiments here described. Radiation affects enzymes definitely, but the effects are not sufficient, it is believed, to account completely for its action upon living cells. Without doubt, the enzymes in the cell receive their share of acceleration or inhibition, as the case may be, but it is not demonstrated that differences in the behavior of exposed cells are to be attributed wholly, or even chiefly, to the effect of the radiation upon cell enzymes.

Gager[6] in his monograph sums up the investigations which had been made upon the effects of radiation previous to 1908. On these points he says, "With only one or two exceptions, exposure to radium rays has been found to either retard or completely inhibit all cell activities," and "Radium rays appear to retard the activity of enzymes."

Not all experiments on animal tissues and products have given uniform results. It has been claimed in certain cases that radium emanations have not affected the enzymes studied. In other cases, for example, the experiments of Henri and Mayer,[7] injury resulted from the exposure. The investigators named found that when invertase, emulsin, and trypsin were exposed to radiation their activity was gradually decreased and finally lost. Still other workers have asserted that radiation favored the action of enzymes. Mention has already been made of the fact that Schwarz found radium rays to decompose the lecithin of the yolk, and he adds that cells rich in lecithin are most sensitive to the rays. Neuberg's experiments lead him to a different conclusion: pure lecithin is not decomposed, and he doubts whether any de-

[6] Gager, C. S.: Effect of the rays of radium on plants. Mem. N. Y. Bot. Garden, vol. 4, 1908.

[7] Henri, V., and Mayer, A.: Action des radiations du radium sur les colloides, l'haemoglobine, les ferments, et les globules rouges. Compt. Rend. Acad. Sci. Paris, 138, 1904.

composition takes place within the cells. But Neuberg with Wohlgemuth observed "acceleration of the autolytic processes by radium radiation" and "Loewenthal and Wohlgemuth have recently proved that the radium emanation is capable of accelerating the action of the diastatic enzyme of the blood, liver, saliva, or pancreas. 'This favorable action is not always observable immediately; very often retardation occurs during the first twenty-four hours, this being gradually neutralized and then replaced, if the experiment is sufficiently prolonged, by an acceleration. In other cases, the emanation produced only inhibition, which was not compensated when the duration of the experiment was extended' " (Euler[8]). Euler further makes the statement that "acceleration of enzyme-action by the emanation has been established with pepsin and trypsin," and finally, "in general, it may be stated that the healing action found by experience to be exerted by radium emanation depends on the activitation of enzymes. The promotion of plant-growth by the emanation is also to be attributed to enzyme-activation."

Before taking up the experiments which it is the purpose of this paper to describe, the physiological relation between the two forms of radiation, X-rays and radium rays, should be pointed out. In general it has been found that results of the same nature are to be expected from the use of these agents; there are, however, discrepancies which seem at first hard to reconcile with this statement. A comparison of the experiments about to be described and the accounts of the experiments with radium shows that these agents are comparable when only a weak exposure to X-rays is under discussion. Weak radiation from the X-ray tube seems to give results similar to those obtained by exposure to radium bromide (unless the preparation of the latter be very pure and of great intensity). The general preference for radium as a means of experiment is perhaps to be explained by this fact. Exposure to X-rays may cause changes so complex that they are not easily analyzed, while an exposure to radium many times as long may not cause very serious effects.

[8] Euler, H.: General Chemistry of the Enzymes. John Wiley & Sons, N. Y.

EXPERIMENTS

In planning the experiments to test the effect of the X-rays on enzymes, pepsin and diastase were chosen because of the ease with which they could be obtained and studied, and because well defined methods have been worked out for testing their action quantitatively. The digestion of starch by radiated and non-radiated solutions of diastase, and of egg albumen by radiated and non-radiated solutions of pepsin were studied.

The experiments with pepsin will be described first. Scale pepsin of 1 to 3000 strength manufactured by Sharp and Dohme was found to give the most reliable results of any obtainable; and as this pepsin contains the minimum amount of impurity in the form of peptones, it "keeps" longer than any other variety I have tried. I have generally used a solution consisting of 3 per cent pepsin (supersaturated), 0.25 per cent hydrochloric acid and 0.3 per cent sodium fluoride in distilled water. According to Vandevelde as quoted by Euler, sodium fluoride is a disinfectant without effect on the activity of pepsin and trypsin. This solution was found to act uniformly and consistently even if kept for as long a period as five or six days. The method employed was the process of Mett, worked out in the laboratory of Pawlow.[9] Fluid egg white is sucked up into a fine glass tube and coagulated at a temperature slightly below boiling point. The tube is then cut up into short pieces which are placed in carefully measured amounts of the fluid to be investigated, and kept at a constant temperature of about 40°C. "After the termination of a certain period, the length of the pieces of the tube, and that of the undigested remains of the proteid columns, are measured off with the aid of a millimeter scale" and a binocular microscope. "The difference gives the length of the digested proteid cylinders in millimeters and fractions of a millimeter." "Test experiments specially carried out have convinced us that the digestion of the proteid column, at least within the first ten hours is directly proportional to the length of the period." "The diameter of the tube, even within wide limits, is without importance, and

[9] See Pawlow: The Work of the Digestive Glands.

the white of the hen's egg is of sufficiently constant composition to be employed as a test object" (Pawlow). The actual amount of the albumen digested may be calculated from the diameter of the tube and the length of the column digested, but since for each experiment tubes having a uniform lumen were used, the absolute amount of digestion may be neglected and only the one dimension, the length of the column digested, need be taken and compared to give the proportionate amounts of the digestion (for in these experiments we are dealing only with ratios). The length of time which the experiment runs may also within wide limits be disregarded, since all the measurements are made at one time and all are under the same conditions, and since bacterial contamination is prevented by the acidity of the solution and by the disinfectant.

Mett's process has been criticized because the area of albumen exposed to the action of the enzyme is relatively small and the solution must depend upon diffusion at the contact surface for renewal. Since however all of the tubes were under exactly the same conditions except that of the difference in radiation, this criticism would not seem to apply to the present experiment.

In my experiment pieces of tubing of 2 mm. (or in some cases 3 mm.) diameter were cut into lengths of 15 mm. and placed each in a test tube containing 5 cc. of the pepsin solution. These were kept at a temperature of 40°C. and each measured separately. Three sets of tubes were run for each experiment. During the early course of the experiments while pepsin of uncertain degree of purity was being used, definite results were difficult to obtain, but since trustworthy pepsin has been obtained, the results have been absolutely uniform and I have no lack of confidence in them.

The following experiment is given as an example of the data from which the conclusions have been drawn. Only one table is cited, although the experiment has been repeatedly set up (more than a score of times) and the results studied. In none of these trials has any results been obtained which cast even the slightest doubt upon the general conclusion. Some trials have served to a certain extent to amplify it; they will also be discussed briefly. It has not been thought necessary by reason of the entire uniformity of the data to give further proof of the proposition,

although the evidence is abundantly at hand. The experiment cited is a type of the series.

A 3 per cent pepsin solution (containing 0.25 per cent hydrochloric acid and 0.3 per cent sodium fluoride) was used as the test solution. Five cc. of the solution were placed in each of three test tubes for a control. Then, after radiation in shallow dishes at a distance of about four inches below the X-ray tube, 5 cc. of the solution were again placed in each of three tubes. This same procedure was repeated for periods of ten, twenty, and thirty minute radiations. The albumen tube, cut as nearly as possible fifteen millimeters long, were then placed one in each tube, and the whole maintained at a temperature of 40°C. The results are given in the following table.[10] The first column indi-

TABLE 1.

		ORIGINAL TUBE LENGTH	LENGTH OF UNDIGESTED ALBUMEN CYLINDERS	DIGESTED ALBUMEN	TOTALS	AVERAGES
		mm.	*mm.*	*mm.*		
Control	1	14.0	8.5	5.5	16.5	5.5
	2	14.5	9.0	5.5		
	3	15.0	9.5	5.5		
4 minutes Radiation	1	14.0	8.0	6.0	18.5	6.166
	2	14.5	7.75	6.75		
	3	14.0	8.25	5.75		
10 minutes Radiation	1	15.25	10.0	5.5	15.75	5.25
	2	15.25	10.25	5.0		
	3	14.0	8.25	5.5		
20 minutes Radiation	1	14.75	9.75	5.0	16.5	5.5
	2	15.0	8.5	6.5		
	3	14.5	9.5	5.0	(15)	(5)
30 minutes Radiation	1	15.0	10.25	4.75	14.25	4.75
	2	14.5	10.5	4.0		
	3	14.5	9.0	5.5		

[10] That the results given could not be due to differences of evaporation, oxygenation, etc., is shown by the fact that the long and short radiations caused qualitative rather than quantitative differences. If these factors had been the cause, the long radiation should have given the same result as the shorter except as to the degree of the effects. Such was not the case.

cates the original length of the tube of albumen as measured under the binocular; the second, the length of the albumen after digestion has progressed for some time; the third column, being the difference between the first two, indicates the amount of egg-white which has been digested, and gives the results to be compared. The fourth and fifth columns are merely the total and average of the three test tubes of each set.

From this table it will be seen that the controls all gave the same amount of digestion. The radiation of short duration accelerated the rate of digestion, for more albumen was digested from these than from the control, although all were under exactly the same conditions except as to radiation. The radiations of longer duration served to slow down the digestion, for in every case except one the amount of albumen digested was less than in the control. The exception is No. 2 of the twenty-minute radiation. From it there was 1.5 mm. more of albumen digested than from the others of the set in which the undigested columns were both exactly 5 mm. in length. This was due, I think, to uneven coagulation of the egg white in the tube, and should probably, therefore, be neglected. There seems to be a tendency also for the greatest radiation to inhibit the activity of the enzyme as measured by the amount of albumen digested in a given time more than those of less duration. The stronger the radiation, the greater the inhibition, would seem to be the rule, yet the data which I have failed to bear this out in entirety, for the results are not uniform upon this point. However, there is without question some tendency in that direction.

The amount of radiation necessary to bring about the inhibition of the activity of the enzyme depends upon the strength of the current, the state of the vacuum of the tube, and other such factors. In one experiment a radiation of five minutes was sufficient to bring on the inhibition. The average amounts digested in this case, were: control, 6.33 mm.; five minute radiation, 5.25 mm.; ten minute radiation, 4.75 mm.; twenty minute radiation 5.16 mm.; thirty minute radiation, 5.33 mm. In another, a radiation of four minutes was sufficiently strong to prevent the

acceleration, but not strong enough to induce the inhibition; in other words, it was in this case a non-effective radiation. Inhibition appeared in this case only with the stronger radiations. Here the totals of albumen digested for each set of three were: control, 7.5 mm.; four minute radiation, 7.5 mm.; ten minute radiation, 5.5 mm.; twenty minute radiation, 5.75 mm.; and thirty minute radiation, 6.75 mm.

Another experiment gave a further interesting demonstration that a weak radiation accelerated the activity of the enzyme. For the radiation of the pepsin solution in this experiment, a very weak current was used causing only a very faint glow to appear about the X-ray tube. This very weak radiation, although continued for a period of half an hour, was inadequate to cause the usual inhibition. It is, of course, true that some of the tubes showed no more digestion than the control tubes, namely, No. 2 of the five minute radiation, and Nos. 1 of the twenty and thirty minute sets; while Nos. 2 of the ten and twenty minute radiations showed less digestion. However, Nos. 1 and 3 of the five minute radiation, Nos. 1 and 3 of the ten minute, No. 3 of the twenty minute, and Nos. 2 and 3 of the thirty minute series showed more digestion of the albumen. The totals and averages of the different series bring out this tendency still more clearly. These facts, taken in conjunction with the data from other experiments as already indicated, are regarded as evidence that a weak radiation, rather than inhibiting the activity of the enzyme, increases it. The data are given in the following table.

These experiments show that an exposure to X-rays according to its intensity, duration, etc., may be classed as accelerative, non-effective and inhibitive. Applied to enzyme reactions an accelerative radiation is one which hastens the speed of the reaction (that is, a weak radiation), while an inhibitive radiation is one following which the speed of the reaction is decreased (a strong radiation). Theoretically, there should be an intensity of radiation between these two which would result in no variation in the reaction from the normal, a non-effective radiation. Such cases have indeed been found to occur, as pointed out above.

The non-effective radiation on the average appears to be of about five minutes duration at four inches distance from the tube, with the apparatus and current used for these experiments.

The experiments as to the effect of radiation on diastase gave results quite similar to those just described for pepsin. It was found convenient to use the preparation known as Taka-diastase for most of the tests. Other earlier experiments on a diastase of less high quality showed the same tendency in their behavior

TABLE 2.

		ORIGINAL TUBE LENGTH	LENGTH OF UNDIGESTED ALBUMEN CYLINDERS	DIGESTED ALBUMEN	TOTALS	AVERAGES
		mm.	*mm.*	*mm.*		
Control	1	19.5	16.5	3.0	8.5	2.833
	2	20.5	18.0	2.5		
	3	20.0	17.0	3.0		
5 minutes Radiation	1	19.75	16.0	3.75	10.25	3.416
	2	20.25	17.0	3.0		
	3	19.5	16.0	3.5		
10 minutes Radiation	1	19.5	16.0	3.5	9.25	3.083
	2	19.5	17.0	2.5		
	3	19.75	16.5	3.25		
20 minutes Radiation	1	20.0	17.0	3.0	9.25	3.083
	2	19.0	16.75	2.25		
	3	20.0	16.0	4.0		
30 minutes Radiation	1	20.0	17.0	3.0	10.5	3.5
	2	20.0	16.5	3.5		
	3	20.0	16.0	4.0		

as did this preparation, but owing to the greater impurity the results were somewhat inconclusive, and were not regarded as trustworthy. These preliminary experiments therefore are not included in those upon which this account is based. A 1 per cent solution of Taka-diastase freshly made at the beginning of each trial and mixed with a 1 per cent solution of chemically pure corn starch was used for most of the experiments. The amount of sugar produced in this mixture was quantitatively determined at frequent intervals during the progress of the reac-

tion by Benedict's Method No. 2.[11] The amount of the mixture necessary to reduce 10 cc. of Benedict's reagent was determined and compared in the case of radiated and non-radiated diastase. In these experiments the starch solution was not radiated (in order to avoid any possible complications which might thus be introduced) but a portion of the solution of diastase was exposed to the X-ray tube at a distance of about four inches for varying lengths of time. Both control and radiated solutions were then mixed at the same time with equal amounts of the starch paste, and within a few minutes the tests for the determination of the sugar content were begun. For every experiment a control was run and the rates of the reactions compared.

Although Taka-diastase was used for most of the experiments described below, the results have been checked up on a diastase of malt prepared by Merck and marked "absolute." The diastase behaves under the influence of X-rays in exactly the same manner as far as could be determined as the Taka-diastase. The same conclusions apply to both.

As in the case of pepsin, two distinct kinds of effects follow exposure to X-rays, depending upon the duration of the exposure: that is an exposure of not more than four or five minutes has the general effect of increasing the activity of the diastase, while one of longer duration decreased it.

If a solution of diastase be mixed with starch and quantitative determinations made at frequent intervals thereafter, it is found that progressively less and less of the mixture is required to reduce a given amount (10 cc.) of the copper sulphate solution used in Benedict's method, due of course to the fact that more and more sugar is produced. If a part of the diastase be radiated before mixing for a length of time not exceeding five minutes, the production of sugar goes forward at a more rapid rate, as shown by the fact that less of the radiated mixture is required to reduce the 10 cc. than of the non-radiated. This experiment has been done repeatedly, and in one case only has a contradictory result been obtained; this exception, since it occurred only once, may probably be explained as due to experimental error. The following table gives the data from a typical experiment.

[11] Hawk: Practical Physiological Chemistry, p. 386.

The Taka-diastase solution used was radiated four minutes, at a distance of four inches from the tube. It, as well as a control solution,[12] was mixed with the starch and the first reading taken five minutes later. The figures indicate the amount of the mixture necessary to reduce the 10 cc. of copper sulphate solution.

TABLE 3.

NON-RADIATED MIXTURE		RADIATED DIASTASE	
	cc.		*cc.*
5 minutes after mixing	2.85	10 minutes after mixing	1.85
+20 minutes	2.30	+20 minutes	1.70
+20 minutes	1.80	+20 minutes	1.45
+50 minutes	1.60	+51 minutes	1.25
+3½ hours	1.30	+3½ hours	1.15

This table shows that since less of the radiated solution was necessary to reduce the given amount of the copper sulphate, more sugar was produced in it in a given time than in the control. The greater amount of sugar produced indicates that the radiation of the diastase solution had the effect of accelerating the enzyme.

A radiation lasting five minutes may prove ineffective as in the case of the pepsin as described above; that is, this amount of radiation is not enough to inhibit the activity, but is sufficient to prevent the acceleration. The exact duration of the non-effective radiation depends of course upon the strength of the current and other such factors.

A longer radiation serves to inhibit the activity of the diastase. These experiments seem to show no special difference in the effects of long radiations of varying duration. A radiation of fifteen minutes inhibits just as effectively as does one of thirty minutes.

Experiments have been made repeatedly to test the inhibitory action of a long radiation, and the evidence obtained is con-

[12] The controls were made as follows: the diastase solution was separated into parts and placed in open flat dishes in the room in which the experiments were performed. One portion was then radiated while the other was shielded from the rays and kept for a control. The conditions of oxygenation, evaporation, etc., were as much as possible the same in the exposed and the control dishes and the radiated solution was modified with respect to the factor under investigation alone. Both solutions were then mixed with starch.

clusive regarding it. The data presented in the following table illustrative of this point are taken from a typical experiment. The diastase used in this experiment was radiated twenty-five minutes.

TABLE 4.

NON-RADIATED DIASTASE		RADIATED DIASTASE	
	cc.		*cc.*
8 minutes after mixing	1.60	15 minutes after mixing	1.90
+13 minutes	1.45	+13 minutes	1.90
+60 minutes	1.30	+65 minutes	1.80
+85 minutes	1.20	+75 minutes	2.00

Since the amount of the mixture necessary to reduce the copper sulphate solution was greater in the radiated than in the non-radiated diastase, less sugar was produced in a given time in the former than in the latter, and the diastase was inhibited by the radiation (for radiation was the only factor with respect to which the two mixtures differed).

In addition to the enzymes mentioned above, a single experiment was performed upon the enzyme produced by *Bacillus prodigiosus*. This organism has the power of liquefying gelatine by enzymotic activity. If the bacilli be removed after the liquefication is complete either by filtering or by some sterilizing agent, the liquefied gelatine may be used to liquefy other gelatine. The effect of radiation on this sterile enzyme was tried, and while the results warrant no definite statement, they seem to be in line with the conclusions given here. This experiment is mentioned merely as suggestive.

SUMMARY

The experiments here described demonstrate that, at least for the digestion of egg albumen by pepsin and of starch by diastase, a short radiation with X-rays has the effect of accelerating the enzymotic activity, while a longer radiation inhibits it. Between these two strengths lies a point at which the radiation is non-effective. Although the accelerative or inhibitive effects may perhaps be slight, they are, nevertheless, definite.

THE TOXICITY OF SODIUM TARTRATE[1]

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Though tartrates are frequently used in medicine and in foods, the knowledge of their action is remarkably fragmentary. The need for more extensive information on this subject was soon recognized by the writers in the course of studies on the pharmacology of the heavy metals, when their salts of tartaric acid were frequently used. Control experiments with sodium tartrate were therefore resorted to. Investigations on the action of the tartrates have been conducted by several workers. Devergie[2] found that doses of 8 to 10 grams of tartaric acid fed to dogs were fatal within one or two hours. Meyer and Steinfeld[3] reported experiments on frogs in which doses exceeding 30 to 60 mgm. when injected into the lymph sacs were fatal. According to Vietinghoff-Scheel,[4] 1 gram of sodium tartrate injected intravenously into a rabbit was rapidly fatal, but his protocol shows that several injections had been made on the same day, before the final dose of 1 gram was administered.

The toxicity of the different isomers was studied by Chabrié,[5] who maintained that the levo is more toxic than the dextro tartrate, the racemic is about one-fourth, while the inactive is about one-fifth of the toxicity of the levo. Karczag[6] reported that the action of the levo tartrates has a greater stimulating effect on the inhibitory and vasomotor mechanism than that

[1] Except when otherwise stated, dextro tartrates were used throughout these experiments.

[2] Devergie: Ann. d. Hyg., 1851, xlvi, p. 432.

[3] Meyer and Steinfeld: Arch. Exp. Path. u. Pharm., 1886, xx, p. 46.

[4] Vietinghoff-Scheel; Arch. int. Pharm. et Ther., 1902, x, p. 145.

[5] Chabrié: Compt. Rend. Acad. Sc., 1893, xxvi, p. 1410.

[6] Karczag: Zeitschr. of Biol., 1909, liii, p. 218.

of the dextro tartrate. His experiments on the isolated heart failed to show, however, any difference in their physiological behavior. According to Chio's[7] experiments, the physiological action of all four isomers is the same.

Recent studies have added considerably to our knowledge of the action of tartrates. After the subcutaneous injection of racemic sodium tartrate in doses of 1 to 1.2 gram per kilo into rabbits and dogs with phlorhidzin diabetes Underhill[8] observed a very marked tubular nephritis, the other anatomical structure of the kidneys being left uninjured. Shortly after the appearance of Underhill's report, we presented before the Society for Experimental Biology and Medicine[9] a preliminary communication on renal disturbances in rabbits produced by sodium tartrate, together with observations on the influence of diet. Our experiments were performed before Underhill's discovery came to our knowledge and are in full agreement with his results. In a second article, Underhill, Wells and Goldschmidt[10] published further experiments on tartrate nephritis which consisted of studies of various factors modifying the action of tartrates. They found that much larger doses were required to produce the same effect when given by mouth than when injected subcutaneously. After 0.5 gram per kilo of Rochelle salts was injected subcutaneously into a well fed rabbit vacuolation of the epithelium of the convoluted tubules and casts were observed. One gram per kilo produced complete necrosis of the epithelium of the convoluted tubules. When Rochelle salts (from 3 to 4 grams per kilo) were given by mouth only slight changes were observed. After larger doses (5 to 5.5 grams per kilo), well marked lesions of the kidney, of the same character as those observed after subcutaneous injection of effective doses, were produced. The changes they state were more marked in fasting rabbits than in the well fed animals.

[7] Chio: Arch. int. Pharm. et Ther., 1912, xxxii, p. 473.

[8] Underhill: J. Biol. Chem., 1912, xii, p. 115.

[9] Salant and Smith: Proc. Soc. Exp. Biol. and Med., 1913, x, no. 5, p. 170.

[10] Underhill, Wells and Goldschmidt, Jour. Exp. Med., 1913, xviii, p. 332, ibid., 347.

The action of alkali, the authors believe, renders the kidney more resistant while phlorhidzin is without effect on the production of tartrate nephritis. Pearce and Ringer,[11] whose experiments were carried out on dogs, found that from 10 to 15 grams of Rochelle salts given intraperitoneally produced marked tubular degeneration of the kidney with some changes in the glomeruli. The subcutaneous injection of 10 grams of Rochelle salts in one experiment and of 10 grams of dextro tartaric acid in another produced tubular nephritis. Seven grams of Rochelle salts by mouth caused death in less than twenty-four hours, the tubules and glomeruli being affected. On the other hand, 15 grams of tartaric acid given in the form of a sodium salt and repeated the next day caused diarrhea but no symptons of nephritis.

In the following experiments attention was directed to the general toxicity of the salts of tartaric acid with special reference to conditions which may modify their action. Diet, the reaction of different animals to the substance, and mode of administration were the factors considered.

EXPERIMENTS ON FROGS

A large number of experiments was carried out, the tests being performed during the months of November and December. Although the resistance of different individuals varied considerably the symptoms produced were the same.

The injection of sodium tartrate into the dorsal lymph sac when given in sufficient quantities was followed by coma, fibrillary twitching, muscular weakness and paralysis of the extremities. After very large doses (10 mgm. of the salt per gram) death usually supervened within two or three hours. The duration of life varied after smaller doses. Symptoms of severe intoxication were constant after 6 mgm. per gram; for some frogs such doses were fatal. This amount may therefore be regarded as the minimum lethal dose, although some frogs resisted somewhat higher doses.

[11] Pearce and Ringer: Jour. Med. Research, 1913, xxix, p. 57.

The levo tartrate was also tested in a number of experiments. The action varied in different individuals, but in general the symptoms and toxic dose were the same as those of the dextro variety. Our results, therefore, contradict those of Chabrié and of Karczag.

The recent studies on the fate of the tartrates in the body are of interest in this connection. According to Brion[12] the levo-tartrate is almost completely burned in the body, while considerable amounts of the other isomers are eliminated unchanged when fed to dogs. Neuberg and Saneyoshi[13] maintained that the decomposition of the several isomers in the body is about the same. The experiments of Underhill, Wells and Goldschmidt[14] would seem to indicate that after subcutaneous injection of sodium tartrate into rabbits, little or none is eliminated in the urine. This they believe is due to renal disturbances caused by the substance.

Experiments on frogs in which the action of sodium tartrate was typical are presented in the following protocols.

EXPERIMENTS WITH DEXTRO TARTRATE

Series XVI-A. Frog 10. Weight, 32 grams

December 12. 10.10 a.m., 1.9 cc. of 10 per cent dextro sodium tartrate (6 grams per kilo) injected.

10.20 a.m., diminished frequency of respiratory movements; fibrillary twitching of the muscles of the extremities, which was more marked in the anterior extremities.

10.30 a.m., fibrillary twitching of muscles in general; frog lying on back; no attempt to move; symptoms of severe intoxication manifested.

December 13, 14, 15, and 16. Condition good.

Frog 11. Weight, 27 grams

December 12. 10.45 a.m., 1.6 cc. of 10 per cent dextro sodium tartrate (6 grams per kilo) injected.

[12] Brion: Zeitschr. f. Physiol., 1898, xv, p. 283.

[13] Neuberg and Saneyoshi: Bioch. Ztschr., 1911, xxxvi, p. 32.

[14] Underhill, Wells, and Goldschmidt: Jour. Exp. Med., 1913, xviii, p. 317.

11.00 a.m., respiratory movements very infrequent, fibrillary twitching present but not general and not marked; frog looked dull; able to turn over when put on back.

2.10 p.m., looked normal; turned over quite readily when placed on back, hence marked improvement; respiratory movements improved but still slow and superficial.

December 13 to 16. Condition good.

Series XVII-A. Frog 1. Weight, 36 grams

December 14. 10.30 a.m., 3.6 cc. of 10 per cent dextro sodium tartrate injected; frog restless immediately after injection; respiratory movement stopped, unconscious, lay on back for about two minutes, then turned over and assumed normal position, but no respiration visible for some time.

10.40 a.m., respiratory movements weak and infrequent.

11.15 a.m., symptoms of severe intoxication.

1.00 p.m., respiratory movements fair, fibrillary twitching of muscles marked.

5.00 p.m., condition good; recovered.

December 15. 10.30 a.m., looked normal.

December 16. 9.00 a.m., dead.

Frog 2. Weight, 38 grams

December 14. 10.35 a.m., 3.8 cc. of 10 per cent dextro sodium tartrate injected; restlessness present but much less than in frog 1; respiratory movements, which stopped for three minutes, returned, but were weak and infrequent.

11.15 a.m., symptoms severe.

1.00 p.m., apparently dead.

2.00 p.m., found dead.

Series XII-A. Frog 4. Weight, 33 grams

November 23. 2.00 p.m., 2 cc. of 10 per cent dextro sodium tartrate injected; restless soon after injection.

2.15 p.m., coma and paralysis; fibrillary twitching of muscles of body and feet very marked.

3.00 p.m., no respiration; heart beats visible through skin.

4.00 p.m., heart was exposed, was chocolate colored, dilated, and still beating.

EXPERIMENTS WITH LEVO TARTRATE

Frog 6. Weight, 35 grams

November 23. 1.45 p.m., 2 cc. (6 grams per kilo) levo tartrate injected into the dorsal lymph sac.

2.00 p.m., fibrillary twitching of muscles of thigh, but not very marked; respiration suspended, a few voluntary movements of posterior extremities now and then; no cardiac movements visible even after skin was removed; when heart was exposed it was found beating, which was probably due to the stimulating action of warm air in the room.

Series XI-A. Frog 3a. Weight, 37 grams

November 21. 12.00 noon, 3.7 cc. of 10 per cent levo sodium tartrate injected.

12.05 p.m., general twitching of muscles of body and legs; made unsuccessful attempts to turn over when placed on back.

1.10 p.m., frog still breathed but paralyzed and in comatose condition.

1.55 p.m., frog dead.

Autopsy. Heart enormously dilated and chocolate colored. Executed one beat once in a while. Mechanical stimulation increased frequency of beats. Continued to beat for three hours.

Frog 4a. Weight, 36 grams

November 21 1.40 p.m., 2 cc. of 10 per cent levo sodium tartrate injected into dorsal lymph sac; a few minutes later frog grew very restless.

1.45 p.m., paralyzed, but still breathed; toes twitched.

2.15 p.m., improvement noticeable; turned over, though slowly, when placed on back.

November 22. 9.00 a.m., looked normal; no impairment of muscular efficiency.

Under observation until November 27; no symptoms.

Series XVI-A. Frog 1. Weight, 46 grams

December 9. 3.10 p.m., 3 cc. of 10 per cent levo sodium tartrate injected.

4.30 p.m., paralysis and coma; respiratory movements not frequent.

December 10. 9.30 a.m., condition good; did not turn over readily, thus showing impaired muscular efficiency; twitching of toes observed.

December 11 to 16. Alive, apparently in good condition.

Series XVI-A. Frog 3. Weight, 50 grams

December 9. 3.20 p.m., 3 cc. of 10 per cent levo sodium tartrate injected.

4.20 p.m., severe muscular twitching; general condition fair, but muscular weakness present.

December 10. 9.30 a.m., general condition good; muscular coördination not impaired, but twitching of toes, especially of posterior extremities, present; no other symptoms.

December 11 to 16. Alive, in good condition.

December 17. Dead.

EXPERIMENTS ON RABBITS

Intravenous injections

When sodium tartrate is introduced directly into the blood stream symptoms of acute intoxication are rapidly developed. As illustrated in experiments 1370 and 1363, 1.25 to 1.5 gram of the crystalline salt in an isotonic or slightly hypertonic solution, injected either into the ear vein or into the jugular vein under ether anesthesia, was sufficient to produce convulsions when the rate of injection was 100 to 200 mgm. of sodium tartrate per kilo per minute. The toxicity was distinctly less, however, when the rate of injection was decreased. The injection of tartrate at the rate of 50 mgm. per kilo per minute, as in experiment 1371, required a dose of 2.2 grams per kilo to induce acute symptoms and 4.2 grams per kilo to cause acute death. In a number of experiments a strong hypertonic solution was used. The data obtained thus far indicate considerable variations in the size of the acute toxic dose, which is not proportional to the speed of injection. In those experiments in which the animal survived the acute effects, or when the dose was a little below the acute minimum toxic dose, severe symp-

toms developed within two or three days. Diminished secretion or complete suppression of urine was observed the next day. The small amounts of urine contained a very large percentage of albumin, and were usually alkaline in reaction, though in some animals it was neutral or acid. On the second or third day twitching of the muscles, fibrillary contraction, muscle tremors and unilateral paralysis, usually of the right side, were observed. The animals sometimes rolled about the long axis of the body, describing a circle as in forced movements. The duration of life was practically from two to eight days. On post mortem examination a sero-fibrinous peritonitis was present, and in a number of cases blood clots and bloody fluid were found in the peritoneal cavity. In some subjects large hemorrhages were also present in the psoas muscles. The pleural cavity contained a variable amount of fluid which was not bloody. The kidney gave in all cases evidence of severe damage. It was very much enlarged in size, and the increase in weight was from 50 to 100 per cent. With smaller doses the nervous or muscular symptoms were not constant. After 0.4 to 1 gram per kilo were injected nervous and muscular symptoms were rare. Evidence of renal disturbance was present when doses of 0.424 to 0.6 grams per kilo were injected. We found that such amounts were seldom fatal, but they invariably produced albuminuria which usually appeared on the third day and lasted approximately two days. About 0.4 gram per kilo may be regarded as the minimum dose which can produce renal disturbance, as 0.24 to 0.3 gram per kilo was without any effect. The reaction of urine was acid in all of the experiments with small doses. It is worthy of note that diarrhea failed to develop in any of these experiments, even after the intravenous injection of 3 grams per kilo.

Rabbit 1370. Weight, 1320 grams

February 28. 1.32 grams per kilo injected into ear veins.

11.00 a.m., 20 cc. of 3.5 per cent sodium tartrate injected in one minute.

11.06 a.m., 10 cc. of 3.5 per cent sodium tartrate injected in three minutes; muscular twitching.

11.10 a.m., received 17 cc. of 3.5 per cent sodium tartrate in three minutes; convulsions, lasted a few seconds.

11.16 a.m., received 3 cc. (total 50 cc.); restlessness, dyspnoea.

March 2. 100 cc. acid urine, considerable albumin present.

March 3. 40 cc. acid urine, albumin much more than on previous days.

March 4. No urine; found dead next morning.

Rabbit 1363. Weight, 1570 grams

February 24. 1.5 grams per kilo injected into ear vein.

11.43 a.m., 10 cc. of 2.5 per cent sodium tartrate injected in two minutes

11.47 a.m., 8 cc. of 2.5 per cent sodium tartrate injected in two and one-half minutes.

11.50 a.m., 10 cc. of 2.5 per cent sodium tartrate injected in two and one-half minutes.

11.55 a.m., 10 cc. of 2.5 per cent sodium tartrate injected in two and one-half minutes.

11.59 a.m., 10 cc. of 2.5 per cent sodium tartrate injected in four minutes.

12.02 p.m., 10 cc. of 2.5 per cent sodium tartrate injected in two minutes.

12.05 p.m., 6 cc. of 2.5 per cent sodium tartrate injected in one minute.

12.10 p.m., 10 cc. of 2.5 per cent sodum tartrate injected in one minute.

12.12 p.m., 10 cc. of 2.5 per cent sodium tartrate injected in one minute.

12.14 a.m., 10 cc. of 2.5 per cent sodium tartrate injected in one minute; convulsions.

12.16 p.m., paralysis.

12.18 p.m., recovered; respiration markedly accelerated; pupils dilated; rabbit lost appetite after injection; secreted 8 cc. of urine in the last two days, which was neutral in reaction, and which contained large amounts of albumin, but no sugar; muscular twitching and a very marked ataxia developed on the second day; rabbit rolled about on long axis of its body; was found dead three days after injection.

Autopsy. Sero fibrinous peritonitis, hemorrhages between peritoneum and psoas muscle. Large amount of fluid in peritoneal and pleural cavity. Lungs consolidated and liver enlarged. Heart dilated.

Rabbit 1344. Weight, 1505 grams

February 28. 2.24 grams per kilo injected into jugular vein under ether anesthesia.

2.59 p.m., 50 cc. of 2.5 per cent sodium tartrate in three minutes.

3.02 p.m., dyspnoea.

3.05 p.m., 40 cc. injected in two minutes; convulsions (after a total of 90 cc. or $1\frac{1}{3}$ gram of sodium tartrate per kilo were injected).

3.07 p.m., 10 cc. injected in one minute; convulsion.

3.08 p.m., 20 cc. injected in one-half minute; respiration stopped; heart beating.

3.08:45 p.m., respiration returned.

3.09:30 p.m., 35 cc. injected in half minute; respiration slow, almost stopped (total 135 cc.)

3.10 p.m., respiration stopped; heart beating; twitching of jaw muscles.

3.11:15 p.m., no heart action.

Autopsy. 3.15 p.m., heart in diastole; weak beats observed.

Rabbit 1371. Weight 1615 grams

February 28. 4.2 grams per kilo injected into jugular vein, under ether anesthesia.

11.43 a.m., 5 cc. of 3.5 per cent sodium tartrate injected in one minute.

11.59 a.m., 39 cc. of sodium tartrate injected in twelve minutes.

12.05 a.m., 11 cc. injected in five and one-half minutes.

12.20 p.m., 50 cc. of 3.5 per cent sodium tartrate injected in fifteen minutes (total 105 cc. or 1.9 per kilo); convulsions.

12.20:30 p.m., fibrillary contraction of muscles.

12.21 p.m., 10 cc. injected; convulsions; respiration suspended 15 seconds later.

12.40 p.m., 50 cc. injected in nineteen minutes.

12.42 p.m., 5 cc. urine obtained from bladder.

12.57 p.m., convulsions; had received 44 cc. in seventeen minutes.

12.58 p.m., respiration stopped.

Autopsy. 12.59 p.m., heart contracting slightly. No fluid in pleural cavity; no fluid in peritoneal cavity; intestines greatly dilated; gall bladder normal; bladder empty; kidneys normal in appearance.

Rabbit 1366. Weight, 1370 grams

February 25. 1.37 gram per kilo injected into jugular vein.

11.33 a.m., 20 cc. of 2 5 per cent sodium tartrate injected in three minutes.

11.38 a.m., 20 cc. of 2.5 per cent sodium tartrate injected in four minutes.

11.50 a.m., 21 cc. of 2.5 per cent sodium tartrate injected in three minutes.

11.55 a.m., struggling observed.

11.58 a.m., struggling observed.

12.00 noon, 14 cc. of 2.5 per cent sodium tartrate injected in four minutes.

Muscle tremor, ataxia, and forced movements were observed two days after injection. Considerable amounts of albumin were present in the urine the next and following days after injection.

Autopsy. Large amount of bloody fluid and blood clots in peritoneal cavity; kidneys weighed 20 grams, mottled in appearance; thoracic cavity contained fluid which was not bloody.

Rabbit 1037. Weight, 1910 grams

June 6. 5 cc. of 10 per cent sodium tartrate (0.3 per kilo) injected into ear vein in five minutes; no symptoms observed on day of injection; examination of urine for albumin and sugar at intervals of forty-eight hours during thirteen days was negative

June 20. Weight, 1630 grams. Passed 55 cc. of acid urine.

10.52 a.m., received 10 cc. of 10 per cent sodium tartrate (0.6 gram per kilo) injected into ear vein.

10.53 a.m., behaved as if asphyxiated; lay flat on belly; reflexes increased very much; pupils dilated; dyspnoea.

10.55 a.m., muscle tremors; ataxia present but not marked.

10.56 a.m., congestion of ears present but not as marked as in case of other rabbits similarly treated.

June 21. Passed 9 cc. of neutral urine; albumin present but no sugar.

June 23. Passed 165 cc. of acid urine; moderate amount of albumin present but no sugar.

June 24. Passed 65 cc. of acid urine; albumin as on previous day but no sugar.

Under observation until July 9 when weight was 1505 grams, but no sugar, albumin, or other symptoms were observed.

Subcutaneous injections

In adult rabbits which had been receiving oats and cabbage, 4 dgm. per kilo was the smallest dose after which any effect was observed. Diarrhea usually developed on the fifth or sixth day, rarely on the second day after the injection. Diminished secretion or suppression of urine was observed during the first twenty-four hours. Urinary secretion, however, was reëstablished on the next day.

A mild albuminuria, temporary in character, was observed in several instances. Out of ten experiments in one series seven proved fatal. The duration of life in two of these was eight to ten days; in the other five it was three to six days. Microscopical examination of the organs of two rabbits which were examined post mortem, showed congestion of the liver and inflammation of the mucosa of the small intestine. Microscopical examination of the kidneys in one case showed moderate congestion and proliferation of the connective tissue cells.

In five experiments of another series with 0.4 gram sodium tartrate per kilo, diminished secretion of urine was the only effect observed and that only in one case. The reaction of the urine was not changed. In these experiments the dextro as well as the levo tartrate was used but no difference in their action was observed. It is worthy of note that these experiments were made in the months of August and October while the other experiments were carried out in November to May, inclusive. In a previous communication[15] from this laboratory attention was called to the effect of season on resistance to drugs, which may account for the difference in toxicity in this case.

In experiments with doses of 0.6 gram per kilo, the diet in some cases consisting of oats, in others of oats and cabbage, given several days or weeks before the administration of sodium tartrate and continued all through the experiment, it was found that although a fair proportion survived such a dose, some effects were observed in practically every case. Thus out of ten rabbits injected, six died, the duration of life being one to six days.

[15] Salant and Rieger: Bull. 148, Bur. of Chem., U. S. Dept. Agr., 1912.

Diminished secretion of urine, the presence of albumin and sometimes sugar were observed in some experiments. Microscopical examination of the kidneys of one of these rabbits that died six days after the injection of sodium tartrate, showed marked congestion, hyaline casts, and degeneration of the tubular epithelium; the glomeruli were compressed; there was also proliferation of the connective tissue cells.

Practically the same results were obtained with doses of 0.8 gram per kilo, the diet being oats, or oats and cabbage. Loss of appetite, loss of weight, albuminuria and in some cases death followed when such amounts were given. The percentage of fatal doses varied between 25 per cent and 75 per cent, the average being a little over 50 per cent. The duration of life in most cases was three to six days. The action with larger doses of sodium tartrate was more marked. Severe injury to the kidney was present in every case, and involvment of other organs was also observed. One to 1.2 grams per kilo were followed by nervous and muscular symptoms, which appeared usually within seventy-two hours and became more pronounced the next day. Diarrhea appeared in several days but was never very marked. In one rabbit which received 1.3 gram per kilo no diarrhea developed at any time. Suppression of urine partial or complete was the rule. It usually followed about twenty-four hours after the administration of sodium tartrate. Sugar appeared in the urine of some rabbits. Post mortem examination revealed the presence of large amounts of bloody fluid in the peritoneal cavity, serous effusion into the thoracic cavity, and a catarrhal condition of the stomach and intestines. The lungs were congested, the heart enlarged and dilated. The liver was congested and in some rabbits very much increased in size. The microscopic appearance of the kidneys presented a picture of various stages of congestion, and degeneration of the tubular epithelium with proliferation of connective tissue cells. Considerable interest attaches to the observations we made with sodium tartrate on rabbits which have been fed carrots for some time previous to the experiment. When 1 gram of sodium tartrate per kilo was injected, no change in the condition of such animals was noticed.

The secretion of urine continued to be abundant and free from albumin; there was no loss of appetite and no indication of nervous or muscular disturbance. After 2.50 grams per kilo, a transient albuminuria was observed, exceptionally however symptoms appeared after smaller doses. The minimum toxic dose is, therefore, about 2.5 grams per kilo. A trace of albumin in the urine was the only effect observed in young rabbits which received 1.6 grams per kilo. Records of our observations with large doses are shown in the following experiments:

Rabbit 936. Male gray. Diet, cabbage and oats. Weight, 2140 grams

November 20. Passed 56 cc. of acid urine.

November 21. Weight, 2095 grams; drank 70 cc. of water, passed 66 cc. of acid urine; received 10 cc. of 20 per cent sodium tartrate (dextro), subcutaneously.

November 22. Weight, 2090 grams; drank 100 cc. of water and passed 21 cc. of acid urine; no symptoms.

November 23. Weight, 2050 grams; drank 46 cc. of water; no urine passed.

November 24. Nervous and muscular symptoms observed.

November 25. Weight, 2040 grams; drank 135 cc. of water and passed 22 cc. of acid urine; head turned to one side; loss of muscular coördination; could not balance himself; rolled on long axis of body as in forced movements; muscular tremor present, restless; succeeded later in raising himself on his legs but remained in one place as if afraid to move; hind legs still, fore legs in motion; when touched described a circle, hind legs as center.

November 26. Weight, 1970 grams; drank 35 cc. of water and passed 37 cc. of acid urine; unable to stand on feet; lay on left side; symptoms more marked than on previous day.

November 27. Found dead 9.00 a.m.

Autopsy. Straw colored fluid in pleural and abdominal cavities; lungs slightly congested; liver showed severe congestion; spleen only slightly congested; kidneys pale, enlarged and petechiated; blood vessels of the small intestine injected.

Rabbit 938. Diet, cabbage and oats. Weight, 2080 grams

November 20. Drank 83 cc. of water and passed 38 cc. of acid urine.

November 21. Weight, 2025 grams; drank 38 cc. of water and passed 44 cc. of acid urine; received 10 cc. of 20 per cent sodium tartrate, subcutaneously.

November 22. Weight, 2050 grams; drank 100 cc. of water and passed 17 cc. of urine; trace of albumin present; no symptoms except small volume of urine.

November 23. Weight, 2010 grams; drank 23 cc. of water; no urine passed; no other symptoms.

November 24. Nervous and muscular symptoms observed but not very marked.

November 25. Weight, 2005 grams; drank 185 cc. of water and passed 14 cc. of alkaline urine; trace of albumin present; head turned as in wry neck; tremors very marked; body swings to right side; symptoms not so marked as in other rabbits similarly treated.

November 26 Weight, 1965 grams; drank 40 cc. of water passed 38 cc. of alkaline urine; diarrhea; nervous and muscular symptoms noted.

November 27. Weight, 1935 grams; drank 15 cc. of water, passed 99 cc. of neutral urine; moderate amount of albumin present.

December 2. Weight, 1520 grams; drank 55 cc. of water, passed 144 cc. of acid urine; little albumin present; slight muscle tremors, but able to walk.

December 3. Weight, 1475 grams; drank 50 cc. of water, passed 33 cc. acid urine; turned around on his side, tremors as in paralysis agitans; fell over when placed on floor, leaned against side of cage; lack of coördination.

December 4. Weight, 1435 grams; drank 30 cc. of water, passed 43 cc. of acid urine; trace of albumin present; head to one side; slight muscle tremors; did not fall when placed on floor but did not move around much.

December 5. Weight, 1375 grams; drank 100 cc. of water, passed 28 cc. of acid urine; no urine obtained from bladder at 9.45 a.m.; condition very weak; died at 10.20 a.m.

Autopsy. 1.35 p.m., animal still warm; liver was pale, yellowish, seemed to be enlarged; cortex of kidney had appearance of fatty degeneration; medulla congested; stomach filled with food; mucous membrane pale, lungs congested; heart normal in appearance.

Rabbit 937. Diet, cabbage and oats. Weight, 1745 grams

November 20. Drank 100 cc. of water, passed 151 of acid urine.

November 21. Weight, 1650 grams; drank 100 cc. of water, passed 121 cc. of acid urine; received 10 cc. of 20 per cent sodium tartrate, subcutaneously.

November 22. Weight, 1715 grams; drank 100 cc. of water, passed 81 cc. of urine; albumin present; few drops of blood present; no symptoms except small volume of urine.

November 23. Weight, 1705 grams; drank 100 cc. of water; feces present for first time; no symptoms except anuria.

November 24. Symptoms observed.

November 25. Weight, 1665 grams; drank 85 cc. of water, passed 2 cc. of alkaline urine; head turned to one side; muscle tremors, almost convulsions when disturbed; symptoms like paralysis agitans; made attempt to walk, body swaying to one side, hind legs spread apart; loss of muscular coördination; trace of diarrhea.

November 26. Weight, 1590 grams; drank 16 cc. of water, passed 16 cc. of acid urine; symptoms more marked than previous day; animal did not walk; head touched floor; diarrhea more pronounced than on previous day.

November 27. 9.00 a.m., found dead.

Autopsy. Peritoneal cavity contained large amount of bloody fluid; liver intensely congested; gall bladder enormously distended with bile, stomach contained hemorrhagic spots but otherwise pale; small intestine injected; pleural cavity contained a good deal of fluid; heart dilated; lungs congested.

Rabbit 1306. White female. Diet, carrots. Weight, 1720 grams

March 9. 9.30 a.m., urine examined; no albumin; no sugar.

March 10. 9.30 a.m., passed 305 cc. of urine; no albumin; no sugar.

11.30 a.m., injected subcutaneously, sodium tartrate, 1.5 gram per kilo.

March 11. Passed 110 cc. of urine; 9 cc. obtained from bladder, neutral, trace of albumin; no sugar.

March 12. Passed 130 cc. of urine, 51 cc. from bladder was neutral and contained a trace of albumin; no sugar; slight incoördination, which disappeared within two days.

March 13. 102 cc. of urine passed, 22 cc. from bladder, neutral and contained a moderate amount of albumin; no sugar.

March 14. 100 cc. of urine passed, 8 cc. from bladder was acid and contained a trace of albumin.

March 15. 355 cc. of urine passed, 24 cc. from bladder alkaline; no albumin; no sugar; appetite which was very poor since tartrate was injected improved very much; under observation until March 19, no albumin was found in the urine nor any other symptoms observed.

Rabbit No. 1346. 2.5 grams per kilo

DATE	WEIGHT	CARROTS	WATER	URINE		REMARKS
	grams	*grams*	*cc.*	*cc.*	*reaction*	
March						
30	1275	650	80	295	alkaline	No albumin; no sugar.
31		300	40	164	alkaline	No albumin; no sugar.
April						
1	1275	350	40	170	alkaline	No albumin; no sugar; received 32 cc. of 10 per cent sodium tartrate.
2		400	55	250		Trace albumin; no sugar.
3		400	50	180		No albumin; no sugar.
4		350	50	170		No albumin; no sugar.
6	1270	775	70	310	alkaline	No albumin; no sugar.
7		325	30	184		Trace albumin; no sugar.
8		225	50	100	alkaline	No albumin; no sugar.
9	1250	295	30	102	alkaline	No albumin; no sugar.

Rabbit 1347. 2.5 grams per kilo

March						
30	1365	550	100	335	alkaline	No albumin, no sugar.
31		210	35	68		Cage urine examined; no albumin; no sugar.
April						
1	1365	335	55	285	alkaline	No albumin; no sugar; received 34 cc. of 10 per cent sodium tartrate.
2		290	65	220		
3		305	40	209		No albumin; no sugar.
4		285	45	176	alkaline	No albumin; no sugar.
6	1330	610	100	290	alkaline	No albumin; no sugar.
7		200	60	124	alkaline	No albumin; no sugar.
8		320	30	188	alkaline	No albumin; no sugar; diarrhea mild.
9	1270	295	70	210		No albumin; no sugar.

Administration of sodium tartrate by mouth

When given by mouth the toxicity was considerably less than by subcutaneous injection. After the administration of 5 grams per kilo, the duration of life was less than twenty-four hours. Doses of 3.5 to 4 grams per kilo failed to produce any effects. Examination of the urine daily for several days did not show the presence of albumin or sugar nor were any other symptoms noticed. Six grams per kilo caused death in one hour in one

rabbit; in another it was fatal within six hours. Diarrhea was present in some cases, in others none was observed after 5 to 6 grams but on post mortem examination the intestines were found enormously distended and filled with fluid feces. Severe diarrhea was observed, however, after doses of 8 to 10 grams per kilo. Muscular twitching and tremors were noticed after large doses and in some rabbits after receiving 6 grams per kilo, but these were not constant symptoms. The reaction of the urine was alkaline in all cases after the administration of sodium tartrate by mouth.

Rabbit 972. Male. Previous diet, oats and cabbage. Weight, 1675 grams

January 14. 10.45 a.m., received 50 cc. of 20 per cent sodium tartrate, dextro, by mouth; exhausted; slight muscle tremor; rapid breathing, bladder squeezed, but no urine; little feces, black and hard; found dead at 11.45 a.m.

Autopsy. 1.30 p.m., intestine enormously distended with fluid, heart dilated; autopsy showed that animal did not die from strangulation.

Rabbit 975. White male. Diet, oats and cabbage. Weight, 2005 grams

January 20. 10.45 a.m., received 50 cc. of 20 per cent dextro sodium tartrate.

4.15 p.m., very slight tremors.

January 21. Found dead 9.00 a.m. Since injection ate 25 grams of cabbage, drank 100 cc. of water; secreted 60 cc. of strongly alkaline urine; there was also slight diarrhea, containing a large amount of carbonates.

Rabbit 948. Previous diet, oats and cabbage Weight, 1535 grams

November 27. Received 30 cc. (4 grams per kilo) 20 per cent sodium tartrate, dextro; under observation for ten days; urine examined was alkaline; no albumin; no sugar.

EXPERIMENTS ON YOUNG RABBITS

The toxicity of sodium tartrate was studied in these experiments on young animals which were fed either carrots or milk exclusively and on suckling rabbits. After the subcutaneous

Experiments upon young rabbits with sodium tartrate. Diet, carrots and milk.

DATE	RABBIT NO.	WEIGHT	DIET	DOSE PER KILO	DURATION OF EXPERIMENT	DURATION OF LIFE	REMARKS
		grams			*days*	*days*	
June							
16	1071	890	carrots	0.8	8		No symptoms.
16	1072	555	carrots	0.8	8		No symptoms.
16	1073	555	carrots	0.8	8		No symptoms.
24	1071-A	990	carrots	1.6	8		Trace of albumin.
24	1072-A	715	carrots	1.6	8		Trace albumin one day.
24	1073-A	730	carrots	1.6	8		Trace albumin on first day.
20	1065	600	milk	0.6	8		No symptoms.
20	1066	760	milk	0.6	8		Trace sugar on day of injection; no other symptoms.
May							
16	1065	255	milk	0.8	13		No symptoms; suckling rabbit.
June							
24	1065	600	milk	0.8	9	11	Perhaps died of starvation; much albumin.
24	1066	680	milk	0.8	9		Much albumin.
24	1067	565	milk	0.8	9	13	Diarrhea and albumin; died of starvation.
	S*	205	milk	1.0	13		Trace of albumin on third day; no other symptoms.
	S3	220	milk	1.0	13		Little albumin on third and fourth days; no other symptoms.
	S4	230	milk	1.0	7		Trace of albumin on second day, only symptom.
	S5	260	milk	1.0	7		Trace of albumin on second day, only symptom.
	S1	180	milk	1.5		2	Muscle tremors.
	S2	205	milk	1.5		7	Muscle tremors.
	SL1	240	milk	1.5		2	Muscle tremors; paralysis; much albumin.
	SL2	210	milk	1.5		2	Muscle tremors; paralysis; no albumin.

*S = suckling rabbit.

injection of 1.6 grams of tartrate per kilo into rabbits which have been receiving carrots, a trace of albumin in the urine on one day was the only effect observed. That the diet per se was not the cause of this slight and transient albuminuria was shown

by the fact that a previous injection of 0.8 gram of sodium tartrate into the same subjects was not followed by albuminuria, the diet being the same in both instances. Sodium tartrate administered to rabbits on a milk diet was much more toxic. Albuminuria which was rather marked in some subjects, and in exceptional cases diarrhea, which subsided after a few days, were the effects noticed after 0.8 gram per kilo. These symptoms were not observed on control days previous to the administration of the tartrate nor when a dose of 0.6 gram per kilo of the salt was given. A milk diet of itself is not the cause therefore of the renal or enteric disturbance. In suckling rabbits a slight albuminuria was observed after the administration of 1 gram per kilo. When the dose was increased to 1.5 grams per kilo nervous and muscular symptoms developed and the animals died two to seven days after the injection. The results with tartrates on a diet of carrots presented above, point therefore to the conclusion that young rabbits are fully as resistant as full grown animals, while half grown rabbits on a milk diet are decidedly less resistant than suckling rabbits. The difference in the reaction when rabbits are fed milk or carrots furnishes additional evidence of the relation of diet to the toxicity of sodium tartrate.

EXPERIMENTS ON CATS

Sodium tartrate was administered by mouth and subcutaneously. Symptoms of gastro-intestinal and renal disturbance were observed after feeding large doses. Ten to 16 grams per kilo produced vomiting and severe diarrhea. A trace of albumin appeared on the third or fourth day which persisted for several days in some subjects but disappeared the next day in others. One cat which received 16 grams per kilo was found dead the next morning although it vomited fifteen minutes after the tartrate was ingested. All the other cats which were similarly treated, survived, no other symptoms being observed. After similar doses, diarrhea, which appeared on the fourth day in one cat, was the only symptom noted. The urine was alkaline

to litmus on the day following the ingestion of 4 grams per kilo, but changed to acid the following day. After 5 grams per kilo it was still alkaline six days later. Neither vomiting nor albuminuria was observed after small doses. The subcutaneous administration of sodium tartrate was found to be much more toxic. Two grams per kilo were fatal in three out of four experiments. One and five-tenths grams per kilo given to three cats caused severe symptoms and were fatal for two cats. The symptoms observed were stiffness, especially of the posterior extremities, marked tremors, convulsions, then paralysis. Reflexes were not increased. Vomiting occurred on the next day. There was no diarrhea at any time. Albumin varying from a trace to a moderate quantity was observed after 1 gram per kilo was injected into two well fed cats, but no other symptoms were observed. The urine was alkaline in one cat for four days after the injection. The alkalinity in the other persisted, being still alkaline at the end of two weeks. The same amount injected into two cats which had been fasting for two weeks previous to the experiments, caused marked renal disturbance as indicated by the presence of large amounts of albumin in the urine which was acid in reaction. No other symptoms were observed. Illustrative protocols are shown in the table on the following page.

EXPERIMENTS ON DOMESTIC FOWLS

The toxicity of sodium tartrate was studied in full grown male birds, usually white Leghorns, weighing 1 to 1.5 kilos. The diet consisted of cracked corn and oats which was given ad libitum. The intramuscular injection of 3 to 4 grams per kilo failed to produce any symptoms in the majority of our experiments. In a few subjects, however (2 out of 10), the dose was fatal. Oedema and marked cyanosis of the comb as in ergot poisoning were observed in these and in other experiments with larger doses. After 6 grams per kilo, coma and paralysis were also observed. This dose was invariably fatal. In no case were symptoms of delayed poisoning observed. Even when tartrate was administered daily for some time in increasing doses, acute

Cat No. 143. Weight, 2960 grams. 2 grams per kilo subcutaneously.

DATE	CONDITION	MEAT	WATER	URINE		REMARKS
January		*grams*	*cc.*	*cc.*	*reaction*	
23						
24	Received 30 cc. of a fresh solution of d. sodium tartrate 20 per cent					
25	Vomited about 5 cc. of white fluid which was neutral to litmus; slight tremors present; awkward gait; stiffness in posterior extremities	15	100	165	acid	Urine turbid; vomited; no sugar; albumin present.
26	Stiffness in hind extremities; awkward gait; seemed improved; no diarrhea; may have vomited, small quantity of greenish fluid in cage (10 cc.)			148	alkaline very slight	Albumin present; no sugar.
27	Condition weaker than on preceding day; unable to walk more than a few steps; ataxia	20	200	52	neutral	Urine turbid; albumin present; no sugar.
28	Awkward gait, easily fatigued; condition seemed improved	10	90	158	acid	Albumin present; no sugar.
29	Nose bloody, unable to walk, condition weaker	none	70	80	acid	
30	Found dead 9.00 a.m.	none	20	70	acid	Albumin, large quantity.

Cat No. 148. Grey and white; weight, 2395 grams.*

April						
28						
29		100	75	57	acid	
30		100	75	104	acid	No feces.
May						
1	Received 48 cc. of 20 per cent sodium tartrate by mouth	100	75	35	acid	7 grams of feces.

2	Vomited		75	159	alkaline	
3		95	75	35	acid	No feces; no albumin; no sugar.
4			75			
5		100†	75	57†	acid	Left part of food; feces in food box; diarrhea.
6		100	75	103	acid	No feces.
7		100	75	107	acid	No feces.
8		100	75	56	acid	12 grams of feces.
9		100	75	385	acid	No feces.
12	Received 18 cc. of 20 per cent sodium tartrate (1.5 grams per kilo subcutaneously)	100	ad lib.	107	acid	13 grams of feces; no albumin; no sugar.
13	No symptoms	45	ad lib.	5	acid	No feces; vomited; little water; no food; no albumin.
14	Awkward gait in hind legs	none	ad lib.	190	neutral	Trace of albumin; no sugar.
15	Ataxia	none	ad lib.	93	acid	No feces; trace albumin; no sugar.
16	Ataxia as before	none	ad lib.	40	acid	Trace of albumin; no sugar; no feces.
17	Condition same as preceding day; animal showed no inclination to walk	none	ad lib.	185	alkaline	No feces; trace of albumin; no sugar.
19	Ataxia, almost complete paralysis	none		180	alkaline	Large amount of albumin; no carbonate; no sugar; no feces.
	Found dead 3.30 p.m.			9	alkaline	Albumin and carbonate present; no sugar.

* Received water by stomach tube.
† Record for two days.

effects only were noticed. It may be remarked that diarrhea was never noticed after sodium tartrate was administered on a grain diet.

FOWL NO.	WEIGHT	DOSE PER KILO	FATAL DOSE	DIET	DURATION OF LIFE	REMARKS
	grams	*grams*	*grams*		*days*	
97	1060	4.0	4.0	corn+oats	5	On first day after injection, bird vomited; comb very dark.
100	1220	4.0	4.9	corn+oats	survived	No symptoms; after eighteen days still alive, and in good condition.
98	1150	6.0	6.9	none	less than 17 hours	Dead 9.00 a.m.
78	1340	6.0	6.0	none		Dead 9.00 a.m.

Fowl 87. Weight, 1390 grams

March 9, 10, 11. Received 27 cc. of 10 per cent sodium tartrate by intramuscular injection; no symptoms; appetite normal.

March 12. Received 46 cc. of 10 per cent sodium tartrate; the next morning comb very oedematous but no other symptom.

March 17. 4 grams per kilo injected as before; no symptoms.

March 18. No symptoms.

March 19. No symptoms; weight, 1480 grams; 3.00 p.m. 74 cc. of 10 per cent sodium tartrate (5 grams per kilo).

March 20. Marked cyanosis of comb, almost black. Looked depressed.

March 21. Recovered.

DISCUSSION

A comparison of the effects of sodium tartrate shows that the resistance of different animals to this substance is apparently subject to considerable variation. It is worthy of note that the frog and the rooster, two forms widely differing in structure and in metabolic activity, stood the largest amounts of sodium tartrate, the difference in dose, in proportion to the bodyweight, being very small. No adequate explanation can be offered at present. The following considerations may, however, be of value. We found that when a sufficient amount of tartrate was given to roosters, symptoms developed soon, ending in either recovery or death. In mammals the action may be rapid or some time

may elapse before the development of symptoms, depending upon the method of administration and the amount introduced. Subacute effects were noticed in the frog also, but these were not so constant as in the rabbit or cat. If due allowance be made for the rate of injection it will be noticed that the difference in toxicity between the rabbit and the fowl is not at all marked. That the heart was the organ chiefly involved was evident from the following observations: In the rooster, the comb became swollen and markedly cyanosed as in ergot poisoning, while in the frog the heart was in diastole and filled with dark blood. Paralysis of respiration was the immediate cause of death in the rabbit, but cardiac action became distinctly weakened and the heart stopped in diastole. Hence the acute effect is chiefly due to action on the circulation and respiration. This may explain the striking similarity in reaction of these widely different forms.

The resistance of the rabbit and cat is interesting. Whether this is due to a difference in the rate of oxidation is unknown at present. There is no evidence of increased elimination into the intestine of the cat as diarrhea was never observed after subcutaneous injection, while in the rabbit diarrhea, though not a constant symptom, was present. Vomiting in cats, observed after subcutaneous injection, may be an important factor in decreasing toxicity.

Attention may also be called to the very marked influence of diet. Thus we have found that sodium tartrate is much less toxic for rabbits which have been fed carrots than for those which have been receiving cabbage or oats of both. The fatal dose for fasting cats was barely toxic for well-fed cats. The difference in the amounts of glycogen in the body under these conditions is of considerable interest in this connection. The greater resistance of carrot-fed rabbits as compared with rabbits receiving a diet less favorable to the accumulation of glycogen strongly suggests a protective action of glycogen against the toxicity of sodium tartrate.[16] Opie and Alford's[17] experiments

[16] The above glycogen hypothesis is tentative. More experiments on the relation of diet to the toxicity of tartrate are in progress in this laboratory.

[17] Opie and Alford: Jour. Am. Med. Assn., 1914, lxii, p. 895.

in chloroform poisoning, showing the protective action of a carbohydrate diet on the liver, are very suggestive. The greater resistance of suckling rabbits as compared with half grown rabbits on a diet of milk may be due to the same cause, as it is well known that larger amounts of glycogen are present in the very young animals than in older individuals.

SUMMARY

I. Sodium tartrate may produce acute and subacute intoxication.

II. In frogs, acute intoxication was observed in most cases.

III. In the domestic fowl, sodium tartrate produced acute effects only.

IV. (a) Acute effects in rabbits were produced when sodium tartrate was given by mouth or when it was injected intravenously. (b) Subacute intoxication was produced by intravenous and by subcutaneous injections, much smaller doses being required. (c) Symptoms of injury to the kidney alone were produced with small doses. (d) With larger doses renal injury was more marked and symptoms of muscular and nervous symptoms were present.

V. Renal injury and symptoms of muscular and nervous disturbances were observed in the cat.

VI. Carrot-fed rabbits were much more resistant than those which were fed oats and cabbage. Young rabbits on a carrot diet are more resistant than on a milk diet. Resistance seems to decrease with age.

VII. The very large doses borne by the fowl and the frog and the large fatal dose required for the rabbit when injected intravenously, may be due to the same mechanism.

VIII. The toxicity of dextro and levo tartrates was found to be the same.

IX. On account of the size of the doses used in the investigation, no definite conclusion can be drawn regarding the physiological rôle of tartrates in foods.